Aflatoxin Handbook

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Grain Inspection, Packers and Stockyards Administration

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Program Handbook

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Aflatoxin Testing

Foreword

The Aflatoxin Handbook has been revised to incorporate changes to the aflatoxin testing program that have evolved since the handbook was last printed on 2-21-92.

This handbook illustrates step-by-step procedures for testing and certifying grain and commodities for aflatoxin. All official inspection personnel authorized or licensed to perform aflatoxin testing shall reference this handbook for procedures.

/s/ David Orr

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GRAIN INSPECTION, PACKERS AND STOCKYARDS ADMINISTRATION
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CHAPTER 1

GENERAL INFORMATION

1.1 PURPOSE

This handbook establishes official procedures for determining aflatoxin in grain and processed grain products, and certifying the official results.

1.2 BACKGROUND

Aflatoxin is a naturally occurring mycotoxin produced by two types of mold: *Aspergillus flavus* and *Aspergillus parasiticus*. *Aspergillus flavus* is common and widespread in nature and is most often found when certain grains are grown under stressful conditions such as drought. The mold occurs in soil, decaying vegetation, hay, and grains undergoing microbiological deterioration and invades all types of organic substrates whenever and wherever the conditions are favorable for its growth. Favorable conditions include high moisture content and high temperature. At least 13 different types of aflatoxin are produced in nature with aflatoxin B1 considered as the most toxic. While the presence of *Aspergillus flavus* does not always indicate harmful levels of aflatoxin it does mean that the potential for aflatoxin production is present.

GIPSA provides aflatoxin testing service as official criteria for corn, sorghum, wheat, and soybeans, as official criteria under the United States Grain Standards Act (USGSA). Testing is also provided for rice, popcorn, corn meal, corn gluten meal, corn/soy blend, and other processed products governed by the Agricultural Marketing Act (AMA).

Aflatoxin testing services are available nationwide, upon request and for a fee, as either a qualitative (screening above or below a threshold determined by the customer) or as a quantitative (actual results in parts per billion) service using several different types of test kits approved by GIPSA.

To further assist the grain industry, GIPSA also provides, on a limited basis, a complex chemical testing method, High Performance Liquid Chromatography (HPLC) testing for aflatoxin. The HPLC testing procedure is performed, upon request, for Board Appeal inspections only. All official aflatoxin testing is performed as prescribed in the GIPSA directive by authorized employees of GIPSA or licensed delegated/designated agency personnel.

Individuals wanting official aflatoxin testing should contact the nearest FGIS field office or delegated/designated agency.

1.3 MANDATORY TESTING

The 1990 Farm Bill (Food, Agriculture, Conservation, and Trade Act of 1990, P.L. 101-624) amended section 5 of the USGSA to "... require that all corn exported from the United States be tested to ascertain whether it exceeds acceptable level of aflatoxin contamination, unless the contract for export between the buyer and seller stipulates that aflatoxin testing shall not be conducted."

1.4 CONTAMINATION LIMITS

The Food and Drug Administration (FDA) has established action levels for aflatoxin present in food or feed. These limits are established by the Agency to provide an adequate margin of safety to protect human and animal health.

FGIS and FDA, having certain related objectives in carrying out their respective regulatory and service functions, have an agreement (Memorandum of Understanding) to assure the most effective possible system for identifying lots of grain, rice, pulses, and food products which exceed the FDA action levels of aflatoxin contamination. Under the provisions of the Memorandum of Understanding (MOU), FGIS and officially delegated/designated agencies report to FDA, on a lot-by-lot basis, each lot (grain, rice, and processed products) that, during the course of an official sample-lot inspection, exceed the 20 ppb FDA action limit.

Listed below are the FDA action levels for aflatoxins in animal feeds.

20 ppb	For corn and other grains intended for immature animals (including immature poultry) and for dairy animals, or when its destination is not known;
20 ppb	For animal feeds, other than corn or cottonseed meal;
100 ppb	For corn and other grains intended for breeding beef cattle, breeding swine, or mature poultry;
200 ppb	For corn and other grains intended for finishing swine of 100 pounds or greater;
300 ppb	For corn and other grains intended for finishing (i.e., feedlot) beef cattle and for cottonseed meal intended for beef cattle, swine or poultry.

Aflatoxin-contaminated corn lots may be reconditioned under the certain conditions established by FDA. (See Attachment)

1.5 APPROVED TEST METHODS

FGIS has approved test kits for use at field testing locations. The Aflacard T20, AflaCup, and Agri-Screen and QuickTox test kits are approved for qualitative analysis of corn. The Aflatest, Fluoroquant, Veratox-AST, Myco✓, RIDASCREEN Fast Aflatoxin Total and RIDASCREEN Fast Aflatoxin SC test kits provide quantitative analysis but can be used for qualitative results. High Performance Liquid Chromatography (HPLC) testing is reserved for quantitative testing at the Technical Services Division (TSD) only.

FGIS APPROVED TEST METHODS			
Method and Test Kit	Approved for		
	Qualitative	Quantitative	Test Kit Range
Aflacard T20 – (R-Biopharm Rhone)	X		20 ppb
QuickTox - (Envirolix)	X		20 ppb
AflaCup - (International Diagnostics Inc.)	X		20 ppb
AgriScreen - (Neogen)	X		20 ppb
Veratox AST - (Neogen)	X	X	5 - 300 ppb
Fluoroquant - (Romer)	X	X	5 - 300 ppb
Aflatest – (Vicam)	X	X	5 - 300 ppb
Myco✓ - (Strategic Diagnostics Inc.)	X	X	5 - 80 ppb
RIDASCREEN Fast Aflatoxin Total - (r-Biopharm)	X	X	5 - 50 ppb
RIDASCREEN Fast Aflatoxin SC - (r-Biopharm)	X	X	5 - 100 ppb

NOTE: The test ranges are for performing an individual analysis with an undiluted sample extract. To obtain accurate results above the test kit range a supplemental analysis must be performed.

Listed in the table below are the test kits that are commonly used for official aflatoxin analysis. Use the table to determine the appropriate test kit(s) to use for testing the listed grain/commodity. For information concerning the testing of mixed grain, contact the Policies and Procedures Branch.

GRAIN/ COMMODITY	TEST METHOD									
	Aflacard T20	AflaCup	Aflatest	Agri-Screen	Fluoroquant	Myco✓	QuickTox	Ridascreen Fast Aflatoxin Total	Ridascreen Fast Aflatoxin SC	Veratox-AST
Corn	X	X	X	X	X	X	X	X	X	X
Sorghum			X		X	X		X	X	X
Wheat			X		X			X	X	X
Soybeans			X		X			X	X	X
Corn Screenings			(*)							(*)
Corn Meal			X		X	X		X	X	X
Corn Germ Meal			X					X	X	X
Corn Gluten Meal			X					X	X	X
Corn/Soy Blend			X		X	X		X	X	X
Corn Gluten Feed			X							
Flaking Corn Grits			X		(*)					(*)
Corn Flour									X	(*)
Corn Bran										(*)
Popcorn			X		X	X		X	X	X
Milled Rice			X		X			X		X
Rough Rice										(*)
Cracked Corn		(*)	(*)	(*)	(*)	(*)		X	X	(*)

NOTE: An X entered into a block denotes that the test kit has been evaluated and approved for the grain/commodity.

The symbol (*) entered into a block denotes that the test kit is under evaluation by TSD for the grain/commodity and is temporarily approved for official use.

1.6 DISCLAIMER CLAUSE

The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

1.7 TESTING SERVICES

Applicants requesting aflatoxin testing must specify whether qualitative or quantitative testing service is desired. If qualitative analysis is requested, the applicant must specify the level desired (e.g., 20 ppb). Three types of aflatoxin testing services are available as follows:

a. Submitted Sample Service.

Analysis based on a sample submitted by the applicant for service.

b. Official Sample-Lot Service.

Analysis based on an official sample obtained and analyzed by official personnel.

(1) Single lot inspection.

Samples may be obtained and tested on either an individual carrier basis or a composite sample basis (maximum of five railcars or fifteen trucks per composite sample).

(2) Unit train inspection under the CuSum Loading Plan.

Unit trains are analyzed on a sublot basis for corn and sorghum and on a composite basis for other grains. Acceptable sublots must conform to contract specifications when "maximum" limits are specified.

When aflatoxin testing is required, samples may be obtained and tested on either an individual carrier basis or a sublot basis. The maximum size sublot for aflatoxin testing is five railcars for unit trains consisting of less than 200,000 bushels, or less than fifty railcars. For unit trains consisting of 200,000 bushels or more, or fifty railcars or more, the maximum sublot size is ten railcars.

(3) Export shiplots

Export shiplots are analyzed on a sublot basis for corn and sorghum and on a composite basis for other grains. Acceptable sublots must conform to contract specifications when "maximum" limits are specified.

(4) Supplemental Testing.

Upon request, supplemental testing may be performed as follows:

Composite samples may be analyzed in addition to the sublot test for corn and sorghum shiplots or unit trains.

(5) Alternate Testing.

Upon request, alternate testing methods may be used, provided that the minimum testing requirements are met. Examples of alternate testing are as follows:

(a) Sublot testing may be used instead of composite sample analysis for grains routinely tested on a composite basis.

(b) Grain shipments may be tested on a component sample basis in lieu of the sublot basis under the provisions of Book III, Inspection Procedures. Components are combined and averaged to determine the sublot result. Acceptable quality will be based on the sublot result as compared to the contracted "maximum" specification.

c. Warehouse Sample-Lot Inspection Service.

Analysis based on an official sample obtained by a licensed warehouse sampler and analyzed by official personnel.

1.8 REVIEW INSPECTIONS

Sections 800.125 and 800.135 of the USGSA permit a review inspection on either official grade/factors or official criteria. When requested, a review inspection for official grade or official factor and official criteria may be handled separately even though both sets of results are reported on the same certificate.

Review inspection services for aflatoxin are provided on either a new sample or the file sample in accordance with the regulations. Board appeal inspection services are limited to the analysis of file samples.

NOTE: Do not consider any excess grain sample as a "new sample" for the basis of testing.

For submitted samples, lots that are certified on an individual carrier basis, and composite samples representing multiple carriers, a maximum of three review inspections (reinspection, appeal, Board appeal) may be performed on the original inspection service.

Only one field review (reinspection or appeal inspection) is permitted for shiplot, unit train, or lash barge material portions when testing is performed on a sublot basis. However, if the applicant requests a review of the entire lot, up to three review levels of service (reinspection, appeal, board appeal) may be obtained for each sublot included in the lot. Inspection results for each review level shall replace the previous inspection result.

a. Reinspection Service.

The laboratory providing original testing services also provides reinspection services. Applicants may request either qualitative or quantitative analysis unless the original test was quantitative. Then, only a quantitative analysis is available.

b. Appeal Inspection Service.

FGIS field offices provide appeal testing services for aflatoxin. Field offices not equipped to provide testing will make arrangements with another FGIS office to provide the most timely service possible. Applicants may request either qualitative or quantitative analysis unless the original or reinspection tests were quantitative. Then, only a quantitative analysis is available. If samples are sent to a field office for analysis, write the words **"AFLATOXIN APPEAL"** in the "Remarks" section of the grain sample ticket and on the back of the mailing tag.

c. Board Appeal Inspection Services.

Board appeal inspection services are limited to the file sample and are provided by the Board of Appeals and Review (BAR) in Kansas City. Applicants may request either qualitative or quantitative analysis unless the original or reinspection tests were quantitative. Then, only a quantitative analysis is available.

The HPLC method is also available for determining aflatoxin in Board appeal samples. The applicant must specify the HPLC method as the desired determination method. Otherwise, the Board appeal inspection will be conducted using the rapid method (test kits).

When sending samples to the BAR, write the words **"AFLATOXIN BOARD APPEAL"** in the "Remarks" section of the grain sample ticket and on the back of the mailing tag.

1.9 QUALITY ASSURANCE PROGRAM

The Technical Services Division (TSD), located at the Kansas City Technical Center, conducts an aflatoxin check sample program for all specified service points and laboratories providing testing services. TSD is responsible for preparing and distributing check samples each quarter to all official aflatoxin testing locations, analyzing check sample results, notifying field locations of any results indicating problems, and releasing a quarterly summary report to all participating laboratories. Field offices are responsible for routine supervision to assure all laboratories in their circuit provide accurate results. The TSD check sample program is designed to test the capability of the official system and to monitor the accuracy of approved testing methods. The check sample program provides limited performance information that can be used to supplement the routine supervision of official personnel performing testing services.

AFLATOXIN SAMPLING AND RECONDITIONING PROCEDURES

1. FDA RECONDITIONING GUIDELINES

The Food and Drug Administration (FDA) will permit reconditioning of aflatoxin-contaminated corn lots at export locations by mechanical cleaning under the following conditions:

a. Only one attempt at reconditioning is allowed. The analytical results from the reconditioned lot will be the final determination for disposition of the entire lot.

b. To assure proper reconditioning, the grain company must mechanically clean the lot at a rate not to exceed 50 percent of the rated cleaner capacity.

c. FGIS must oversee the cleaning process, sample the reconditioned lot (cleaned corn) using a diverter-type mechanical sampler, and analyze the samples for aflatoxin.

d. FGIS must sample the cleanings/screenings using the most practical procedures available and test the cleanings and/or screenings for aflatoxin contamination.

At interior locations, the local FDA office may modify the reconditioning procedures to provide for a cost effective process.

2. FGIS RESPONSIBILITIES

When positive lots are identified at export locations, field office managers (FOM) should work with the grain facility representatives and develop a standard operating procedure (SOP) for reconditioning aflatoxin-contaminated corn.

FOM's should review the SOP with local FDA officials before implementing the reconditioning process, unless instructed otherwise by FDA.

a. Export Locations

At export locations, FGIS or official delegated state agency personnel, as applicable, are responsible for:

(1) Reporting actionable lots to the local FDA field office.

(2) Preserving the identity of actionable lots prior to reconditioning.

(3) Monitoring the reconditioning process at the grain facility.

(4) Sampling and testing reconditioned lots (cleaned corn and screenings) for aflatoxin. When sampling screenings, use the most practical method available to obtain a representative sample.

(5) Preserving the identity of reconditioned lots and screenings. (Screenings are not considered a reconditioned lot.)

(6) Reporting aflatoxin results of reconditioned lots and screenings to FDA.

(7) Completing a report of the reconditioning process. Include in the report the following information:

(a) Date Reconditioned.

(b) Grain Elevator/Location.

(c) Type of Sample/Carrier.

(d) Original Results.

(e) Reconditioned Whole Grain Results.

(f) Cleanings/Screenings Results.

(g) Size of Cleaner Screens used to Recondition the Lot.

(h) Elevator Set-up Information.

b. Domestic Locations

FOM's servicing interior locations should contact the local FDA office servicing the area where the contaminated lot is located to discuss and determine responsibilities for managing the reconditioning process. Official agencies and affected grain companies are encouraged to participate in these discussions to facilitate the development of an SOP.

3. SAMPLE SIZE AND PREPARATION

Obtain the minimum sample size as directed in chapter 2 of this handbook. If requested by the applicant, a larger sample size may be obtained.

Grind the entire corn sample obtained for aflatoxin testing and prepare three 500-gram subportions from the ground sample.

Sample Portion	Use
Test Portion	Original inspection service
File Portion	Review inpection service
FDA Portion	Retain for FDA analysis if results exceed 20 ppb.

When reconditioned lots are resampled in accordance with the FDA guidelines, a file portion is not required.

If FGIS's original results for a reconditioned lot of corn or screenings exceed 20 ppb, the FDA sample portion will be used for any subsequent verification (by FDA) of results.

4. DISPOSITION POLICY

The grain industry must comply with FDA policy regarding the disposition of corn and screenings resulting from the reconditioning process. In general, disposition will occur as follows:

- Cleanings/screenings may be used for animal feed if the aflatoxin content meets FDA feed guidelines. The screenings may not re-enter food channels in any fashion.

- Reconditioned (cleaned) corn with less than 20 ppb aflatoxin may be handled without restrictions. When the reconditioning process fails and the corn continues to exceed the 20 ppb level, disposition is based on current FDA policy.

Contact the local FDA office regarding other questions concerning specific disposition action.

CHAPTER 2

LABORATORY SAFETY

2.1 GENERAL INFORMATION

Aflatoxin testing requires the use of flammable liquids and suspected carcinogens. The building owner (private or GSA) must permit the use of methanol in space used by FGIS. FGIS will provide testing services onsite only in facilities that provide adequate protection to FGIS personnel. The following space requirements apply to FGIS occupied space only.

2.2 APPROVED FGIS LABORATORY SPACE

Individual elevators may provide two kinds of space for personnel to perform onsite aflatoxin testing. The space may be located (1) in a building along with other occupants or (2) in a building devoted exclusively to laboratory space.

In either case, the plan for the intended laboratory space is subject to inspection and approval by FGIS prior to construction. The Safety and Health Office, Equipment Branch, and field office manager will review proposed plans and suggest ways to comply with the requirements.

The following are minimum requirements for laboratory space:

a. Location.

Locate the laboratory at least 100 feet from the base of the elevator headhouse. This distance is subject to negotiation when the elevator uses exterior grain legs and/or inclined belts in lieu of interior grain legs or where the headhouse is equipped with blow-out panels or the headhouse consists of a lightly covered framework.

Laboratories must meet the following requirements when they are located in a building with other occupants.

(1) Isolate the laboratory from nonlaboratory occupants using a fire barrier having at least a 1-hour fire resistance.

(2) Provide a fire barrier consisting of floors, ceilings, and interior walls.

(3) Provide all passageways and other openings that lead to adjacent interior space with self-closing fire doors having a 1-hour fire resistance. Do not block these doors open.

(4) Separate the space from central heating, ventilation, and air-conditioning using automatic-closing fire dampers in the heating, ventilation, and air-conditioning ducts near the fire barrier, or provide a separate heating, ventilation, and air-conditioning system for the laboratory.

b. Size.

Dedicate the space strictly for laboratory (chemical) work. Supply adequate space for chemical analysis (minimum of 100 square feet) and a separate area for sample preparation and grinding purposes. Samples must be ground in space separate from the analytical space.

c. Electrical System.

Provide the laboratory space with electrical power and lighting meeting the standards of the National Electrical Code. Wiring suitable for a Class I location is not required. A three-wire system consisting of an energized wire, a neutral wire, and a grounding conductor is satisfactory.

Install overhead lighting fixtures through ceilings that serve as fire barriers. Fixtures suspended below such ceilings are acceptable.

d. Exhaust System.

The exhaust system must remove methanol vapors from the work area. Normal air conditioning and heating may provide adequate ventilation when performing testing procedures in a building devoted exclusively for laboratory space. The local Collateral Duty Safety and Health Officer and the Safety and Health Office in Washington, DC, will assist in assessing on a case-by-case basis whether added ventilation, such as a fume hood, is needed. If needed, situate the laboratory space so that hoods, to be supplied by FGIS, are vented to the exterior of the building. Fume hood ventilation will require a 6 or 8 inch diameter opening either vertically through the ceiling and roof or horizontally through an exterior wall. In some cases, a portable hood may be sufficient.

e. Plumbing.

Provide the laboratory space with a basin having hot and cold potable water and a sewer connection.

For further information about these requirements, contact the FGIS Safety and Health Staff.

f. Eyewash and Safety Shower Station.

Provide the laboratory space with eyewash equipment (eyewash bottle or permanent faucet-mounted fixture). A permanent, faucet-mounted eyewash fixture is highly recommended. A safety shower station must be installed in laboratories where acetonitrile-based extraction solvent (Romer-Fluoroquant test method) is used.

2.3 FGIS LABORATORY PRACTICES

When working in a laboratory, FGIS employees must comply with the Chemical Hygiene Plan developed for the laboratory where the testing is performed. To accomplish this, include the following as part of an overall FGIS laboratory "Standard Operating Procedure" (SOP). Maintain the SOP, this handbook, and the current Material Safety Data Sheets (MSDS) and Chemical Hygiene Plan at each laboratory.

a. Label all bottles and containers according to the Hazard Communication Program. In addition, when preparing mixtures of solutions, securely apply a label with the name of the solution, the preparation date, and the preparer's initials written in permanent ink.

b. Do not smoke, eat, drink, or chew gum or tobacco in the laboratory.

c. Wash hands immediately before and after eating, drinking, and smoking outside of the laboratory area.

d. Wear a disposable, fire-retardant laboratory coat and disposable impermeable gloves when working.

e. Clean the laboratory equipment and dispose of contaminated materials according to procedures listed in this chapter.

f. Wear an FGIS-approved disposable mask and hair protection when grinding samples, or when otherwise exposed to airborne grain dust.

g. Do not wear contact lenses in the laboratory if the testing process involves chemicals other than methanol (e.g., acetonitrile).

h. Wear FGIS-approved safety glasses or splash goggles when in the lab (also applies to visitors in the lab).

i. Do not store food or drink in the laboratory refrigerator. Store only the test kits and other items requiring refrigeration.

j. Do not wear protective clothing outside the laboratory unless waste chemicals are being removed to outside storage facilities or extra chemicals are being carried into the laboratory from an outside storage cabinet.

k. Do not store masks and hair protectors in the grinding area where they might become contaminated by the dust particles.

2.4 STORING CHEMICALS AND SOLVENTS

a. Store chemicals and equipment outside the fume hood.

b. Store chemicals in places where they will not clutter bench tops or obstruct movements. Do not store solutions at a height exceeding eye level. Large bottles shall be stored no more than two feet above ground level.

c. Prepare all solutions and perform analyses in a working fume hood.

d. Limit the total quantity of waste chemicals in the laboratory to 1 liquid gallon.

e. Maintain a current MSDS for each chemical at the laboratory. If each supply of chemicals received does not have a MSDS enclosed, contact the company and request one immediately.

f. Limit the total amount of flammable solvent in the laboratory to 2 gallons.

g. Store flammable solvents in an approved solvent storage cabinet.

2.5 CLEANING LABWARE

a. Negative Tests (# 20 ppb).

(1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used glassware, funnels, beakers, etc., wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Place materials in a garbage bag for routine trash disposal.

b. Positive Tests (> 20 ppb).

(1) Labware.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water (e.g., 100 ml bleach to 1,000 ml water). Completely submerge the used glassware, funnels, beakers, etc., and soak for at least 5 minutes. Remove items from the bleach/water solution, submerge in a dishwashing liquid/water solution, wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water in a plastic pail labeled "bleach solution". Soak disposable materials, such as used columns, cuvettes, vials, test kit components, etc., for at least 5 minutes. Pour off the liquid down the drain and place the materials in a garbage bag and discard.

2.6 CLEANING AFLATOXIN SOLUTION SPILLS

Perform the following procedures only while wearing disposable impermeable gloves and chemical splash goggles. If hands become contaminated, wash immediately with undiluted bleach followed by soap and water.

Clean areas and materials contaminated by any aflatoxin solution or positive (i.e., > 20 ppb) extraction solutions spills with bleach. The affected area should be completely covered with 5-6 percent sodium hypochlorite (household bleach) dispensed from a plastic wash bottle or spray bottle. Apply 10 parts of bleach to 1 part of spilled material and leave for at least 5 minutes. Wipe up the bleach using an absorbent cloth or paper towels. Place cleaning materials in a plastic waste bag, close tightly, and discard.

2.7 DISPOSING EXCESS SAMPLE EXTRACT

a. Negative Samples (# 20 ppb).

Dispose of the excess sample extract solution in an approved waste container. Place the sample slurry in a garbage bag for routine disposal.

b. Positive Samples (>20 ppb).

The sample extract and slurry left over after completion of the test procedure must be decontaminated prior to disposal in a waste drum. Once the sample analysis has been completed, using a plastic wash bottle, add bleach to the slurry and allow to filter. When the filtration is complete, dispose of the slurry in a garbage bag.

To decontaminate the sample extraction solution, add bleach equal to one half of the volume remaining in the test tube. Pour the decontaminated extraction solution into an approved waste container.

2.8 WASTE DISPOSAL

Proper disposal of hazardous waste is required by law. The Environmental Protection Agency (EPA) establishes specific guidelines; however, additional local and State laws exist in some locations. It is important that the procedures used for disposing of waste chemicals comply with the laws required at each location.

Contact the local EPA office for disposal information and names of certified waste disposal companies in the area.

a. Chemicals and Solvents.

(1) Dispose of waste according to existing local, State, and Federal laws.

(2) Select an EPA approved or certified waste disposal company in the area. The company must be able to identify the type of waste drum required, provide information regarding sample profile and waste manifest requirements, and provide estimated costs for pick-up based on the results of the sample profile.

(3) Locate waste drums in an area outside the laboratory space that complies with local fire and EPA codes. Label and date waste drums properly.

(4) Post disposal procedures at each laboratory site.

(5) Maintain accurate records with documentation from the disposal company of pick-up and delivery of the waste drums to the waste disposal site.

b. Decontaminated Materials.

Place decontaminated materials, such as filters, test kit components, and disposable lab materials into a garbage bag and dispose of in a dumpster or landfill disposal site. Only the materials that have been decontaminated may be transported. (Do not transport flammables or contaminated materials.)

c. Other.

Label excess ground corn/other grains remaining after aflatoxin testing and ground corn/other grains from official aflatoxin file samples representing grain with greater than 20 ppb: "FOR LABORATORY USE ONLY - NOT FOR USE AS FOOD OR FEEDSTUFF" and dispose of in a dumpster or landfill site.

2.9 FIELD OFFICE MANAGER RESPONSIBILITY

a. Supplement this handbook with an SOP for each testing laboratory. The SOP should be tailored to accommodate the individual workload and environment for each location.

b. Develop a Hazard Communication Program for personnel that perform tests involving hazardous materials and ensure that all personnel complete the program.

c. Contact an EPA-approved or EPA-certified waste disposal company and make arrangements for removal of chemical wastes or provide other suitable waste disposal procedures consistent with existing laws that do not create a hazard to the community.

d. Provide impermeable metal containers meeting Underwriters Laboratory approval for Class I liquids that can be tightly sealed and which are labeled "Flammable" or "Biohazardous Material" or both, as applicable, for storing waste chemicals (e.g., methanol, acetonitrile) and solutions for removal.

e. Provide plastic disposal bags for disposal of decontaminated material such as filter paper, laboratory coats, disposable pipette tips, gloves, etc.

f. Provide containers and labels for disposal of excess grain. Labels are to state "FOR LABORATORY USE ONLY - NOT FOR USE AS FOOD OR FEEDSTUFF," and are to be placed on containers prior to disposal.

g. Provide signs for the laboratory door as follows:

(1) "Biohazardous Material Present."

(2) "No Smoking, Eating, or Drinking."

(3) "Flammable Material Present."

(4) "Wear Safety Protection."

(5) "Admittance of Authorized Personnel Only."

h. Provide signs for the refrigerator, if present, as follows:

(1) "Biohazardous Material Present."

(2) "No Food or Drink to be Stored in This Refrigerator."

i. Provide adequate training for laboratory employees prior to performance of laboratory functions to include:

(1) Information conveying operations and conditions which can result in exposure to aflatoxin.

(2) Contents and availability of Material Safety Data Sheets for relevant chemical agents.

(3) Precautions to take when working with aflatoxin contaminated products, including personal hygiene, personal protection equipment, and methods of decontamination.

(4) Purpose, proper care, and limitations of dust masks and other protective equipment.

(5) Engineering and work practice controls including cleaning methods.

(6) Review of the SOP at the laboratory.

(7) Proper handling and disposal of waste.

j. Maintain the following safety and health records:

(1) Records of any employee injury or illness involving over-exposure to chemicals (29 CFR 1904; 29 CFR 1960.66 through .77b).

(2) List of employees trained and assigned to perform aflatoxin tests.

(3) Copies of any safety and health studies pertaining to the laboratory.

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CHAPTER 3

SAMPLE PREPARATION

3.1 GENERAL INFORMATION

The manner in which samples are obtained and processed is an important consideration when testing for aflatoxin. To ensure that the test results accurately reflect the aflatoxin concentration present in a lot, samples must be representative of the lot and of sufficient size to compensate for the uneven distribution of the contaminant.

3.2 SAMPLE SIZE

Obtain samples according to the instruction in the Grain Inspection Handbook, Book I, "Grain Sampling."

The minimum sample size is based on the type of lot. Applicants may request a sample size larger than the minimum sample size.

Lot Type	Minimum Sample Size (lbs.)/ grams
Trucks	2 pounds / approximately 908 grams
Railcars	3 pounds / approximately 1,362 grams
Barges/Sublots	10 pounds / approximately 4,540 grams

NOTE: A minimum sample size of 10 pounds is required for composite type samples (e.g., a single sample representing multiple carriers). A 10-pound sample size is also recommended, but not required, for submitted samples.

3.3 WORK RECORDS

Each testing laboratory must maintain work records for each test that include the name of the applicant, date of service, sample or carrier identification, test results, initials of official personnel performing the test, and any other information deemed necessary to properly certificate the test results and bill the applicant. As practical, use existing forms, such as FGIS-992, "Services Performed Report;" FGIS-920, "Grain Sample Ticket;" or FGIS-921, "Inspection Log," to record laboratory results.

Any sample sent to TSD (including the Board of Appeals and Review) for aflatoxin testing or monitoring purposes must include the necessary information to facilitate sample processing and testing.

3.4 SAMPLE PORTIONS

a. Subportions.

Grind the entire sample obtained for aflatoxin testing and prepare two 500-gram subportions from the ground sample: A 500-gram work portion for original testing services and a 500-gram file sample portion for review testing. For submitted samples, retain as large a sample as possible.

For sublot testing of corn at export locations, save an additional 500-gram file (three 500-gram subportions total) for Food and Drug Administration (FDA) analysis.

From the 500-gram work portion, divide (using a Boerner divider) out a portion of 50 grams for aflatoxin testing and weigh on an FGIS-approved type scale with a minimum division size of 0.1 gram.

b. Saving File Samples.

Maintain file samples (including the FDA file sample when applicable) for all lots/samples that:

do not meet the contractual specification of the applicant for service;

are required for the aflatoxin monitoring program; or

exceed FDA action limits of 20 ppb.

When applicable, maintain a representative file sample for each lot, sublot, composite, or submitted sample tested. For submitted samples that are less than 500 grams, retain as large a sample as possible. For information concerning file sample retention periods refer to FGIS Directive 9170.13, "Uniform File Sample Retention System".

c. Storing File Samples.

If file samples are required, store each sample in a manner that will maintain the representativeness of the sample and prevent possible manipulation or substitution. Place the sample in paper bags or envelopes and label each file sample with the test date and identification. Take precautions to ensure that file sample containers are strong enough to prevent loss of sample integrity when storing samples. Do not store samples near heat, windows, or in direct sunlight. (Store samples in cold storage if available.)

d. Disposition of File Samples.

At the end of the retention period, label the file samples as follows: "FOR LABORATORY USE ONLY - NOT FOR USE AS FOOD OR FEEDSTUFF," and discard the file samples in a dumpster or landfill disposal site.

e. Shipping Samples.

When it is necessary to send samples to other laboratory locations, take precautions to maintain sample integrity by securely packaging the samples. Label the shipping container "NOT FOR HUMAN CONSUMPTION".

3.5 OPERATION OF GRINDERS

Samples must be ground to a fine particle size that is sufficiently fine enough to obtain a homogeneous blend. Avoid over-grinding or pulverizing a sample because it produces an excessively powdery mix that will slow down the filtration process.

Grinding must be performed in an area separate from the testing area. Use the Romer Mill - Model 2A, Bunn Grinder, or equivalent to grind the sample.

FGIS employees must follow the manufacturer's safety procedures for operating the grinder and must wear protective equipment (i.e., labcoat, mask, gloves, and hairnet) when grinding samples.

a. Romer Mill.

(1) General Operating Instructions.

The Romer Mill simultaneously grinds and subsamples corn at the rate of approximately 1 pound per minute. An adjustable restrictor door located above the collection chute varies the amount of ground sample allowed into the collection chute. Official personnel must adjust the grinder to obtain the required testing and file portions from the sample.

Adjust the grinder by locating the first line (far left) etched on the restrictor door. Position the door approximately 1/3 of the way between the first and second line. For a 10-pound sample, approximately 500 grams will be collected through the collection chute.

Once the grinder is adjusted to obtain the 500-gram sample, mark the location of the setting. To increase the sample size, move the restrictor door to the left.

If a composite sample is required in addition to the sublot-by-sublot analysis, adjust portion sizes as needed to obtain an adequate size composite and still maintain individual file samples. Obtain the composite sample from the ground sublot samples.

(2) Grinding the Sample.

Grind the entire 10-pound sample with the grind lever set at the finest range.

NOTE: Samples with moisture content of 20 percent or more may cause the grinder motor to overheat and the breaker switch to release. If this occurs, allow the motor to cool and then set the grind lever to the coarsest setting by turning it counterclockwise. Do not grind high moisture samples on the fine grind setting.

b. Bunn Grinder.

(1) General Operating Instructions.

The Bunn-O-Matic grinds corn at a rate of approximately 2 pounds per minute and has a holding capacity of approximately 3 to 4 pounds when fully closed. Official personnel must grind the entire sample (see section 3.2) and cut it down (using an FGIS-approved divider) to obtain the required testing and file portions from the sample.

(2) Grinding Samples.

Grind the entire 10-pound sample with the grind lever set at the fine selection. Add 3 to 4 pounds at a time into the hopper until all 10 pounds are ground. If the grinder is experiencing difficulty (e.g., over-heating, bogging down) at the fine setting, change the setting to coarse. After grinding the remainder of the sample at the coarse setting, switch the setting back to fine. Collect the entire 10-pound portion and regrind at the fine setting.

NOTE: Samples with moisture content of 20 percent or more may cause the grinder motor to overheat and the breaker switch to release. If this occurs, allow the motor to cool and then set the grind lever to the coarse setting. Do not grind high moisture samples on the fine grind setting.

3.6 CLEANING GRINDERS

A small amount of ground sample will remain in the grinder after the total sample has been ground. To prevent the contamination of subsequent samples, clean the grinder using one of the following cleaning procedures:

a. If a Vacuum Cleaner is Available.

After a sample has been ground and collected, with the unit turned on, use a vacuum cleaner with an attachment that will fit over the mouth of the chute. Place the attachment at the bottom of each chute for about 30 seconds. After all the chutes have been cleaned, turn the power off and prepare for the next sample.

b. If a Vacuum Cleaner is Not Available.

Clear the grinder by discarding a small portion (first 10 to 15 grams) of the next sample to be tested.

(1) Pour the sample into the grinder and turn it on long enough to collect the first 10 to 15 grams.

(2) Turn the power off, and discard the 10-15 grams ground sample.

(3) Turn the power back on and finish grinding the sample to collect the remaining subsample for analysis.

3.7 CHECKING PARTICLE SIZE

a. Procedures for Checking the Performance of the Grinder.

For locations that perform mycotoxin testing on coarse (e.g., corn) and small grains, perform the check using a 100-gram sample portion of corn using the following procedures.

(1) Grind a sample portion of approximately 100 grams of corn having a moisture content of 14.0 percent or less.

(2) Weigh the entire portion that was ground.

(3) Sieve the portion across a standard No. 20 wire woven sieve.

(4) Weigh the portion that passed through the sieve.

(5) Determine the percent of fine material, by weight, as follows:

Fines = weight from step (4) divided by the weight from step (2) X 100.

b. Optimum Particle Size.

The optimum range for particles of coarse and small grain passing through the No. 20 sieve is between 60 and 75 percent. Whenever the ground particles appear to be too coarse, or the results of a grinder check indicate that less than 50 percent of the ground portion passes through the No. 20 sieve, the grinder should be adjusted or repaired to meet the optimum range requirements.

Grinding apparatuses must be checked periodically to determine whether they are producing a final product that meets the particle size requirements as listed above. Official personnel shall determine the frequency of the checks based on a number of items that include visual observation of the ground product, number of samples ground since last check, and time (number of days) since the last check was performed. Record all particle check results in a convenient location for future reference purposes.

CHAPTER 4

CERTIFICATION

4.1 BACKGROUND

Testing performed on standardized grains (e.g., corn, wheat) is performed as an official criteria factor under the authority of the United States Grain Standards Act (USGSA), as amended. Testing performed on processed grain products (e.g., corn meal) and other commodities is provided under the authority of the Agricultural Marketing Act (AMA) of 1946, as amended.

Aflatoxin results are recorded on the pan ticket, worksheet, or loading log and in the remarks section of the certificate.

Certify aflatoxin test results on grain in accordance with the USGSA/AMA (as applicable) regulations.

Upon the request of the applicant, separate certificates may be issued for grade and for aflatoxin when both are determined on the same lot.

Sections 800.125 and 800.135 of the regulations under the USGSA permit a review inspection on either official grade/factors or official criteria. When requested, a review inspection for official grade or official factors and official criteria may be handled separately, even though both sets of results are reported on the same certificate. When official grade or official factors and official criteria are reported on the same certificate, the review inspection certificate shall show a statement indicating that the review results are for official grade, official factors, or official criteria, and that all other results are those of the original, reinspection, or appeal inspection results, whichever is applicable.

4.2 GENERAL PROCEDURES

The type of service requested and the test method used determine how aflatoxin results are recorded and certified.

a. Qualitative Testing.

(1) Record the results of a **qualitative service** on the pan ticket and inspection log as being equal to or less than a threshold (e.g., 20 ppb) or as exceeding the threshold.

(2) If a **quantitative method** is used to provide qualitative service, record the test results on the work records in a quantitative measurement (e.g., 10 ppb) or a qualitative measurement (e.g., $\leq$ 20 ppb).

(3) Certify results as being equal to or less than a threshold.

b. Quantitative Testing.

Record the results on the pan ticket and the inspection log to the nearest whole ppb.

When test results indicate that aflatoxin is present at a level of less than 5 ppb, certify the results as "Aflatoxin does not exceed 5 ppb."

Certify test results that are between the lower testing range (5 ppb) and the upper testing range (e.g., 80 ppb) to the nearest whole ppb.

Test results greater than the upper testing limit of the test kit are certified as exceeding the test kit range unless a supplemental analysis is performed. For example: An aflatoxin test result of 110 ppb obtained using an aflatoxin test kit with a test range of 5 - 80 ppb would result in the following certification statement: "Aflatoxin exceeds 80 ppb."

c. Certifying Test Results of Single and Combined Lots, Unit Trains, and Shiplots.

(1) Single Lot Inspection Basis for Trucks and Railcars.

Certify each test result on a separate certificate.

(2) Combined Land Carrier Basis for Trucks and Railcars.

If an applicant requests aflatoxin testing on a composite basis (up to 5 railcars and 15 trucks) and the inspection for grade on the basis of individual carriers, factor only certificates are issued for the aflatoxin testing and separate grade certificates are issued for each carrier.

(3) Composite Sample Testing for Shiplots.

Certify the composite results using the appropriate statement.

(4) Submitted Sample Testing.

Certify the results using the appropriate statement.

(5) Unit Train and Shiplot Inspection under the CuSum Loading Plan.

(a) Sublot Size for Shiplots.

The testing frequency for shiplot grain will be the same as the sample for grade analysis unless the applicant specifically requests aflatoxin analysis on the basis of a component sample.

(b) Sublot Size for Unit Trains.

The maximum size sublot for aflatoxin testing is 5 railcars for unit trains consisting of less than 200,000 bushels, or less than 50 cars. For unit trains consisting of 200,000 bushels or more, or 50 railcars or more, the maximum sublot size is 10 railcars.

For unit trains, the sublot size for aflatoxin testing and for grade analysis may be different. For example, an applicant may request grade analysis on the basis of a sublot containing two cars and request aflatoxin analysis on the basis of five cars.

(c) Recording Test Results.

Aflatoxin test results of sublot samples taken throughout loading are recorded on the loading log. A material portion occurs if the sublot result exceeds the limit as specified in the load order.

(d) Certifying Test Results.

Certify the lot based on the mathematical/weighted average (as applicable) of the accepted sublot results.

Certify material portions separately.

(e) Material Portions.

If a material portion occurs, the applicant has the option of requesting a review inspection. Review inspection results replace previous results when determining if a material portion exists.

If a material portion designation due to aflatoxin is not removed by the review inspection process, the applicant may leave the material portion on board and receive a separate certificate; return the grain to the elevator; or discharge the material portion along with additional grain in common stowage equivalent to one half the material portion quantity.

4.3 STANDARD CERTIFICATION STATEMENTS

Use one of the applicable statements for certifying aflatoxin.

a. Qualitative Testing.

When aflatoxin results are equal to or less than a specific threshold (e.g., 20 ppb) ppb:

"Aflatoxin equal to or less than 20 ppb."

"Aflatoxin exceeds 20 ppb."

b. Quantitative Testing.

(1) When aflatoxin results are less than 5 ppb, use the following statement.

"Aflatoxin does not exceed 5 ppb."

(2) When aflatoxin test results are between 5 ppb and the upper testing range (e.g., 80 ppb) of the test kit, round to the nearest whole number in ppb.

"Aflatoxin (result rounded to the nearest whole number) ppb."

(3) When aflatoxin test results exceed the upper testing range (e.g., 300 ppb) of the test kit.

"Aflatoxin exceeds (enter upper test limit) ppb."

c. HPLC Testing.

TSD performs HPLC testing for total aflatoxins, and, upon request of the applicant for service, testing can be performed to measure individual (i.e., B1, B2, G1, G2) aflatoxin isomers in a sample. The limits of quantification for individual aflatoxin measurements are 1 ppb for aflatoxins B1 or B2, and 2 ppb for aflatoxins G1 or G2.

Use the following statement to certify total aflatoxins.

> "Aflatoxin (record actual results to the nearest whole number) ppb. Results based on High Performance Liquid Chromatography Method."

To certify a specific aflatoxin in a sample use the following statement.

> "Aflatoxin (insert B1, B2, G1, or G2 as applicable) (insert result to the nearest whole number) ppb. Results based on High Performance Liquid Chromatography Method."

To certify individual aflatoxins and the total aflatoxin concentration in a sample use the following statement.

> "Aflatoxin (insert B1, B2, G1, or G2 as applicable) (insert result to the nearest whole number) ppb, total aflatoxins (record actual results to the nearest whole number) ppb. Results based on High Performance Liquid Chromatography Method."

4.4 OPTIONAL STATEMENTS

a. Aflatoxin Not Detected.

At the request of the applicant, use the following statement when aflatoxin is not detected (0 ppb).

> "Aflatoxin not detected."

> **NOTE: If sublot results are combined and averaged and the lot average is equal to 0 ppb, but an individual sublot result exceeds 0.0 ppb, the statement may not be used.**

b. Converting to Parts per Million (ppm).

At the request of the applicant, convert and certify the ppb result to parts per million (ppm) using an approved statement. To convert ppb to ppm, divide the ppb result by 1000.

"(Actual ppb result) ppb is equivalent to (converted ppm results) ppm."

c. Converting to Milligrams (mg) per Kilogram (kg), or Micrograms (µg) per Kilogram (kg).

At the request of the applicant, convert and certify results in milligrams per kilogram (mg/kg) or micrograms per kilogram (µg/kg). Use the following equivalents to determine mg/Kg or µg /kg:

ppm = mg/kg ppb = µg /kg

"(Actual ppb result) ppb is equivalent to (converted mg/kg or µg /kg result)."

d. Multiple Results on the Same Certificate.

When certifying multiple aflatoxin results on the same certificate and the results are based on different sample types, the certificate must reflect the difference. As a guideline, the multiple results are shown as follows:

"Sublot sample results: Aflatoxin equal to or less than 20 ppb."

"Composite sample result: Aflatoxin 14 ppb."

e. Negative Result Statement.

At the request of the applicant, one of the following statements may precede the applicable standard statements when test results are equal to or less than 20 ppb.

"The aflatoxin result is negative." OR "Negative aflatoxin."

f. Type of Test Statement.

At the request of the applicant, use this statement to indicate the type of aflatoxin test used.

"Results based on (indicate type of test used) method."

NOTE: These certification statements may be modified as deemed necessary.

4.5 REVIEW INSPECTION STATEMENTS

Use the appropriate statements listed below for reinspection, appeal, and Board appeal inspections.

a. Results are reported on the same kind of certificate issued for the original service and supersede the previously issued inspection certificate.

"This certificate supersedes Certificate No. (number) dated (date)."

b. The superseded certificate is null and void as of the date of the subsequent (reinspection/appeal/Board appeal) certificate.

"The superseded certificate has not been surrendered."

c. When a file sample is used, enter the following statement on the reinspection/appeal/Board appeal certificate:

"Results based on file sample."

d. When reporting more than one official result on the same certificate but at different levels of inspection, explain this condition using one of the following applicable statements:

"(Grade, factor, or official criteria) results based on (new/file) sample. All other results are those of the original inspection service."

"(Grade, factor, or official criteria) results based on the appeal inspection. All other results are those of the (original inspection/reinspection) service."

"(Grade, factor, or official criteria) results based on the Board appeal inspection. All other results are those of the (original inspection/ reinspection/appeal inspection) service."

CHAPTER 5

AFLACUP TEST KIT

5.1 GENERAL INFORMATION

The AflaCup test kit is an enzyme linked immunosorbent assay (ELISA) developed by International Diagnostics Systems Corporation and marketed by Romer Labs. As the name implies, antibodies which react specifically with aflatoxins are contained in a cup. The test provides qualitative (less than or equal to a specified threshold) results.

5.2 PREPARATION OF EXTRACTION SOLUTION

The extraction solvent used in the AflaCup test method is a methanol/water (distilled or deionized) mixture consisting of 80 percent methanol (Reagent grade or better) and 20 percent water.

a. Using a graduated cylinder, measure 800 ml of methanol and place it into a clean carboy with spigot.

b. Add 200 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed.

c. Label the container stating the mixture (80 percent methanol and 20 percent water), date of preparation, and initials of technician who prepared the solution.

d. Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 8 parts methanol to 2 parts of deionized or distilled water.

5.3 EXTRACTION PROCEDURES

a. Transfer 50 grams of ground sample into an extraction mixing jar.

b. Add 100 ml of the (80/20) methanol/water extraction solvent.

c. Cover the extraction jar and blend on high speed for 1 minute.

d. Remove the cover and funnel the extract through a Whatman No.1 filter or a coffee filter into a sample jar labeled with the sample identification.

e. After collecting the filtrate, remove the funnel, filter, and ground material and place over an empty collection container.

5.4 REAGENT CHECK

a. Stabilization.

Prior to performing the test, allow one hour for all reagents to reach room temperature (73E- 84EF).

b. Testing Reagents.

Each day, before testing official samples, test at least one negative control cup to ensure that all reagents are functional.

Use the following procedures to test the control:

(1) Apply 2 drops of negative control (green cap) to the center of the AflaCup.

(2) Using a timer, allow the cup to set for a 1-minute reaction time.

(3) Apply 2 drops of the aflatoxin enzyme (red cap) to the center of the cup.

Note: The enzyme solution may only be used with the antibody-coated cups contained in the same test kit.

(4) Using a timer, allow the cup to set for a 1-minute reaction time.

(5) Wash with 30 drops of the Wash Solution (white cap). When using more than one AflaCup, wash each cup with 3 series of 10 drops per cup.

(6) Prepare fresh Substrate Solution in a small test tube by mixing 10 drops of Substrate Solution A (yellow cap) with 10 drops of Substrate Solution B (blue cap) for each AflaCup.

(Do not combine Substrate Solution A with Substrate Solution B more than 10 minutes before use.)

Note: If a blue color develops immediately after combining Substrates A and B, repeat this step. If the problem persists call Romer Labs for technical assistance.

(7) Add the entire contents of the Substrate Mix from each test tube to each test cup in use.

(8) Using a timer, allow the cup to set for a 1-minute reaction time.

(9) Immediately read and interpret the result.

c. Interpreting Results.

A blue color indicates the reagents are functional. If the color remains white for at least one minute the reagents are not functional and must be replaced.

5.5 TEST PROCEDURES

a. Procedures for a 20 ppb cut-off.

(1) Transfer 200 microliters (µl) of the dilution buffer to a culture tube (12x 75 mm) and then add 100 µl of the filtered extract.

(2) Mix well and slowly apply 100 µl of the mixture to the center of the AflaCup.

(3) Using a timer, allow the cup to set for a 1-minute reaction time.

(4) Apply 2 drops of the aflatoxin enzyme (red cap) to the center of the cup.

Note: The enzyme solution may only be used with the antibody-coated cups contained in the same test kit.

(5) Using a timer, allow the cup to set for a 1-minute reaction time.

(6) Wash with 30 drops of the Wash Solution (white cap). When using more than one AflaCup, wash each cup with 3 series of 10 drops per cup.

(7) Prepare fresh Substrate Solution in a small test tube by mixing 10 drops of Substrate Solution A (yellow cap) with 10 drops of Substrate Solution B (blue cap) for each AflaCup.

(Do not combine Substrate Solution A with Substrate Solution B more than 10 minutes before use.)

Note: If a blue color develops immediately after combining Substrates A and B, repeat this step. If the problem persists call Romer Labs for technical assistance.

(8) Add the entire contents of the Substrate Mix from each test tube to each test cup in use.

(9) Using a timer, allow the cup to set for a 1-minute reaction time.

b. Interpretation of Test Results.

(1) Negative (equal to or less than 20 ppb).

The sample is considered equal to or less than 20 ppb when the cup color changes to blue.

Note: Color will be concentrated in the center of the cup.

(2) Positive (Greater than 20 ppb).

The sample is considered greater than 20 ppb when the cup color remains white for at least one minute.

5.6 REPORTING AND CERTIFYING TEST RESULTS

a. Report results on the pan ticket and inspection log as being equal to or less than a threshold (e.g., 20 ppb) or as exceeding the threshold.

b. Certify results as being equal to or less than a threshold.

c. Refer to the Certification section of the handbook for more detailed certification procedures.

5.7 CLEANING LABWARE

a. Negative Tests (# 20 ppb).

(1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used glassware, funnels, beakers, etc., wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Place materials in a garbage bag for routine trash disposal.

b. Positive Tests (> 20 ppb).

(1) Labware.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water (e.g., 100 ml bleach to 1,000 ml water). Completely submerge the used glassware, funnels, beakers, etc., and soak for at least 5 minutes. Remove items from the bleach/water solution, submerge in a dishwashing liquid/water solution, wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water in a plastic pail labeled "bleach solution". Soak disposable materials, such as used columns, cuvettes, vials, test kit components, etc., for at least 5 minutes. Pour off the liquid down the drain and place the materials in a garbage bag and discard.

5.8 WASTE DISPOSAL

a. Negative Results (# 20 ppb).

If the test result is negative (equal to or less than 20 ppb), discard the filter paper and its contents (ground material) into a plastic garbage bag for disposal. Dispose of any remaining liquid filtrate in the chemical waste container.

b. Positive Results (> 20 ppb).

If the result is positive (more than 20 ppb), the ground portion remaining in the filter paper must be decontaminated prior to disposal. After disposing of the remaining filtered extract in the chemical waste container, filter approximately 50 ml of bleach through the filter containing the ground portion and allow to drain. Discard the filter paper and its contents (ground portion) into a plastic garbage bag for disposal. The bleach rinse filtrate collected may be treated as a non-hazardous solution and disposed of by pouring down the drain.

5.9 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits:

(1) Cups with Aflatoxin Antibody attached.

(2) Aflatoxin Enzyme, Dropper Bottle.

(3) Negative Control Solution, Dropper Bottle.

(4) Wash Solution, Dropper Bottle.

(5) Substrate A, Dropper Bottle.

(6) Substrate B, Dropper Bottle.

(7) Dilution Buffer for Samples, Translucent.

b. Materials Required but not Provided:

(1) Sample grinder.

(2) Balance.

(3) Methanol - Reagent grade or better.

(4) Distilled or deionized water.

(5) Blender with mixing jars.

(6) Cuvette rack.

(7) Pipettor and tips - 100 to 1000 µl adjustable.

(8) 100 ml graduated cylinder.

(9) Funnel.

(10) Timer.

(11) Whatman No.1 Filter Paper or Coffee Filters.

(12) Glass cuvettes (12 x 75 mm).

5.10 STORAGE CONDITIONS

Test kits should be refrigerated between 36E- 46EF.

CHAPTER 6

RIDASCREEN® FAST AFLATOXIN TOTAL TEST KIT

6.1 GENERAL INFORMATION

The RIDASCREEN® FAST Aflatoxin Total test is a competitive enzyme immunoassay for the quantitative analysis of aflatoxin in select grains and commodities. **The test kit is limited to providing aflatoxin measurements between 5 – 50 ppb.** Accurate aflatoxin measurements above 50 ppb can be obtained by performing a supplemental analysis involving a diluted extract.

6.2 PREPARATION OF EXTRACTION SOLUTION

The extraction solvent used in the RIDASCREEN® FAST Aflatoxin Total test is a methanol/water (distilled or deionized) mixture consisting of 70 percent methanol (ACS grade or better) and 30 percent water.

a. Using a graduated cylinder, measure 700 ml of methanol and place it into a clean carboy with spigot.

b. Add 300 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed.

c. Label the container stating the mixture (70 percent methanol and 30 percent water), date of preparation, and initials of technician who prepared the solution.

d. Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 7 parts methanol to 3 parts of deionized or distilled water.

6.3 EXTRACTION PROCEDURES

a. Transfer 50 grams of ground sample into an extraction mixing jar.

b. Add 250 ml of the (70/30) methanol/water extraction solvent.

c. Cover the extraction jar and blend on high speed for 2 minutes.

d. Filter the extract through a filtering syringe.

e. Dilute 1 ml of the filtrate with 1 ml of distilled/deionized water.

6.4 TEST PROCEDURES

a. Sample Analysis.

(1) Allow reagents, microwells, and sample extracts to reach room temperature prior to running the test.

(2) Insert a sufficient number of wells into the microwell holder for all standards and samples to be tested. (For example: to test 11 samples use 16 wells - 5 for the standards and 11 for the test samples).

Test Strip #1

Well #	1	2	3	4	5	6	7	8
Sample	C 0	C 4	C 10	C 20	C 50	S1	S2	S3

Test Strip #2

Well #	1	2	3	4	5	6	7	8
Sample	S4	S5	S6	S7	S8	S9	S10	S11

Where C 0 is the zero control, C 4 is the 4 ppb control, C 10 is the 10 ppb control, C 20 is the 20 ppb control, and C 50 is the 50 ppb control. S1 is sample 1, S2 is sample 2, S3 is sample 3, etc.

NOTE: Do not run more than 3 strips (19 samples) per set of control standards.

(3) Using a new pipette tip for each standard and sample, pipet 50 µl of standards and prepared sample to separate wells.

(4) Add 50 µl of enzyme conjugate (red capped bottle) into each well.

(5) Add 50 µl of anti-aflatoxin antibody (black capped bottle) into each well.

(6) Mix thoroughly by gently sliding the plate back and forth on a flat surface.

(7) Incubate for 5 minutes (± 0.5 minutes) at room temperature.

(8) Dump the contents of the wells. Turn the wells upside down and tap out on a paper towel until the remaining liquid has been removed.

(9) Using a wash bottle, fill each well with distilled or deionized water. Empty the wells again and remove all remaining liquid. Repeat this step 2 times (total of 3 washes).

(10) Add 100 µl of substrate/chromagen (white dropper bottle) to each well.

(11) Mix thoroughly by gently sliding the plate back and forth on a flat surface.

(12) Incubate for 5 minutes (± 0.5 minutes) at room temperature (64 – 86º F). Cover the wells with a paper towel to protect them from light sources.

(13) Add 100 µl of stop solution (yellow or orange dropper bottle) to each well.

(14) Mix thoroughly by gently sliding the plate back and forth on a flat surface.

(15) Measure absorbance at 450 nm using the Biotek EL 301, or Awareness Technology Stat-Fax Model 303 PLUS microwell readers.

(Results must be read within 10 minutes)

b. Reading Results with the Microwell Reader.

(1) Biotek EL 301 Microwell Reader.

(a) Make sure that the microwell reader is on and allowed to warm-up for a minimum of 15 minutes before using.

(b) Remove sample carriage and hit "Enter."

(c) Insert W2 filter and hit "Enter."

(d) Insert W1 filter (450 nm) and hit "Enter."

(e) Hit "Clear" and then "Blank." This will cause the instrument to read air as the blank sample.

(f) Load microwells into sample carriage so that the first control labeled 0 is in position A1.

(g) Load the sample carriage into the strip reader so that position A1 is under the light beam of the reader.

(h) Press "Read" and an absorbance value for A1 should appear in the display on the microwell reader. Record the value.

(i) Slide the carriage to position A2 and press "Read." An absorbance value for A2 will appear. Record the value.

(j) Repeat step (i) until absorbance values have been obtained for all controls and samples. Record the values.

(k) Use the RIDA®SOFT Win Data software provided by r-Biopharm to convert the absorbance values into concentration values.

(2) Stat-Fax Model 303 PLUS Microwell Reader

(a) To begin from the "Ready" prompt, press Menu, key in the test number, and then press Enter.

(b) The screen will read, "Set carrier to A, press enter." Place the wells all the way to the right in the carrier. Push the carrier all the way to the left to line up the notch with the wells, then press enter. The carrier will advance into the reader, and it should start to print.

(c) When the reader is finished reading the strip, the screen will read, "Plot Curve Y/N?"

Press "Yes" (1/A) to print the graph,

Press "No" (0) to skip this feature.

(d) The screen will read, "Accept Curve Y/N ?"

Press "Yes" (1/A) to accept the curve and proceed to read another strip. When finished reading the second strip, press "Clear" twice and the results strip will print, "Test Ended."

Press "No" (0) to end the test.

6.5 REPORTING AND CERTIFYING TEST RESULTS

a. Report all results on the pan ticket and the inspection log to the nearest whole ppb.

b. Sample results over 50 ppb are reported as >50 ppb unless a supplemental analysis is performed.

c. Refer to the Certification section of the handbook for more detailed certification procedures.

6.6 SUPPLEMENTAL ANALYSIS

a. Diluting the Sample Extract.

If quantitative results are above the testing limits (i.e., 50 ppb) of the test kit, test results are reported as exceeding the limit. To determine and report an aflatoxin level higher than 50 ppb, the sample extract must be diluted so that a value between 5 and 50 ppb is obtained.

The final aflatoxin concentration is calculated by multiplying the results with the diluted extract by the dilution factor.

b. Example.

If the original analysis reported the aflatoxin value at 70 ppb, the sample extract would be diluted using the following procedures in order to obtain a true value.

(1) Dilute 5 ml of the original extract with 5 ml of the extraction solvent mixture. The total volume is 10 ml. This is a 1 to 2 dilution (compares volume in the beginning with the total volume in the end).

(2) Proceed to sample analysis .

(3) Multiply the analytical results obtained by 2 to obtain the actual aflatoxin concentration. For example, if 34 ppb was the original value obtained with the diluted extract, the actual concentration in the original sample was 68 ppb.

$$\text{True Aflatoxin Value} = \frac{\text{Total Volume}}{\text{Initial Extract Volume}} \times \text{Aflatoxin Result}$$

$$\text{True Aflatoxin Value} = (10 \div 5) \times 34 \text{ ppb}$$
$$= 2 \times 34 \text{ ppb} = 68 \text{ ppb}$$

6.7 CLEANING LABWARE

a. Negative Tests (≤ 20 ppb).

(1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used glassware, beakers, etc., wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Place materials in a garbage bag for routine trash disposal.

b. Positive Tests (> 20 ppb).

(1) Labware.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water (e.g., 100 ml bleach to 1,000 ml water). Completely submerge the used glassware, beakers, etc., and soak for at least 5 minutes. Remove items from the bleach/water solution, submerge in a dishwashing liquid/water solution, wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water in a plastic pail labeled "bleach solution". Soak disposable materials, such as used columns, cuvettes, vials, test kit components, etc., for at least 5 minutes. Pour the liquid down the drain and place the materials in a garbage bag and discard.

6.8 WASTE DISPOSAL

a. Negative Results (≤ 20 ppb).

If the test result is negative (equal to or less than 20 ppb), discard the syringe into a plastic garbage bag for disposal.

b. Positive Results (> 20 ppb).

If the result is positive (more than 20 ppb), the remaining ground portion must be decontaminated, using bleach, prior to disposal. Discard the filter syringe and remaining ground portion into a plastic garbage bag for disposal. The bleach rinse filtrate collected may be treated as a non-hazardous solution and disposed of by pouring down the drain.

6.9 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits

(1) 1 microtiter plate.

(2) 48 antibody coated microwells.

(3) 5 aflatoxin standard solutions of 1.3 ml each; 0, 4, 10, 20, and 50 ppb aflatoxins.

(4) 1 red-capped bottle of 3 ml peroxidase conjugated aflatoxin solution.

(5) 1 black-capped bottle of 3 ml anti-aflatoxin antibody.

(6) Microwell holder.

(7) 1 white dropper bottle of 6 ml Substrate/Chromagen.

(8) 1 yellow or orange dropper bottle of Stop reagent.

b. Materials Required but not Provided:

(1) Methanol - ACS grade or better.

(2) Deionized or Distilled Water.

(3) 250 ml graduated cylinder.

(4) 125 ml container.

(5) Filtering syringe (JM1000).

(6) Sample collection tubes.

(7) Waring high-speed blender with a one liter jar, or equivalent.

(8) Sample grinder.

(9) Balance.

(10) Biotek EL 301 or an Awareness Technology Inc. Stat-Fax Model 303 Plus Microwell reader equipped with a 450-nm filter.

(11) Eppendorf Repipettor, or equivalent, and 2.5 ml syringes.

(12) 50 µl and 1000 µl Pipettor and pipette tips.

(13) Paper towels, Kaydry paper or equivalent absorbent material.

(14) Waste receptacle.

(15) Timer: 3 channel minimum.

(16) Waterproof marker, Sharpie or equivalent.

(17) Wash bottle.

(18) Deionized or distilled water.

6.10 STORAGE CONDITIONS

a. Storage Conditions.

(1) The reagents supplied with the test kit can be used until the expiration date on the kit label when stored refrigerated at temperatures between 35° F and 46° F. **(DO NOT FREEZE)**

(2) Return any unused microwells to their original foil bag and reseal them together with the desiccant provided.

(3) The substrate/chromogen solution is light sensitive, therefore, avoid exposure to direct light.

b. Indication of Instability or Deterioration of Reagents.

(1) Any bluish coloration of the red stained substrate/chromogen solution is indicative for deterioration and the reagent should be discarded.

(2) A value of less than 0.6 absorbance units for the zero standard may indicate deterioration of reagents.

CHAPTER 7

AGRI-SCREEN TEST KIT

7.1 GENERAL INFORMATION

The Agri-Screen test is a sequential competitive enzyme immunoassay that provides qualitative (equal to or less than a specified threshold) results.

7.2 PREPARATION OF EXTRACTION SOLUTION

The extraction solvent used in the Agri-Screen test method is a methanol/water (distilled or deionized) mixture consisting of 70 percent methanol (ACS grade or better) and 30 percent water.

a. Using a graduated cylinder, measure 700 ml of methanol and place it into a clean carboy with spigot.

b. Add 300 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed.

c. Label the container stating the mixture (70 percent methanol and 30 percent water), date of preparation, and initials of technician who prepared the solution.

d. Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 7 parts methanol to 3 parts of deionized or distilled water.

7.3 EXTRACTION PROCEDURES

a. Standard Procedure.

(1) Transfer 50 grams of ground sample into an extraction mixing jar.

(2) Add 250 ml of the (70/30) methanol/water extraction solvent.

(3) Cover the extraction jar and blend on high speed for 2 minutes.

(4) Remove the cover and funnel the extract through a Whatman No.1 filter or a coffee filter into a sample jar labeled with the sample identification.

(5) After collecting the filtrate, remove the funnel, filter, and ground material and place over an empty collection container.

b. Alternate Procedure.

(1) Transfer 50 grams of ground sample into a whirlpack bag.

(2) Add 250 ml of the (70/30) methanol/water extraction solvent to the bag and secure tightly.

(3) Shake the sample portion and extraction solvent vigorously by hand for 3 minutes.

(4) Let the slurry stand for 1 minute, then pour off a small amount of the extract from the bag into the filter paper mounted over the collection container.

(5) Close the bag securely and save until ready for waste disposal.

7.4 TEST PROCEDURES

a. Preparation of Solutions.

(1) Remove aluminum seals from blue-labeled, red-labeled, and yellow-labeled bottles and set aside.

(2) Substrate is pre-activated and is ready for use. Substrate should be stored in the dark. Remove only one vial of substrate at a time from the foil pouch prior to use.

b. Sample Analysis.

(1) Select a test kit that has stabilized/warmed to room temperature 68° - 82° F for 1-hour prior to use.

(2) Open a foil bag and remove 2 red-marked mixing wells and 2 antibody-coated wells for each sample to be tested. Place in the microwell holder and mark the left end of the strip with a "1". Return unused strips to the foil package and close tightly.

Document the identification of all antibody-coated sample wells in order to identify the wells after washing.

	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12
mixing wells	O	O	O	O	O	O	O	O	O	O	O	O
	C	S1	C	S2	C	S3	C	S4	C	S5	C	S6

"W" = well number e.g., #1 through #12
"C" = control
"S1, S2, S3, & S4" = sample numbers

Where C is the 20 ppb control, and S1, S2, etc. are the sample numbers

(3) Firmly place a pipette tip on the 100 microliter (µl) pipettor/syringe and add one preset amount of the Enzyme Conjugate (blue-labeled bottle) into each mixing well (priming pipette tip first). Discard pipette tip.

NOTE: When dispensing any test liquids, prime pipette tip by drawing liquid up into the tip and dispensing it back into the bottle one or two times.

(4) Firmly place a pipette tip on the 100 µl pipettor/syringe and add one preset amount from the 20 ppb Control (yellow-labeled bottle) to the first mixing well of the red-marked strip. Discard the pipette tip.

If testing more than one sample, place 100 µl of 20 ppb Control into mixing well #3 (second sample), mixing well #5 (third sample), etc.

(5) Firmly place a new pipette tip on the 100 µl pipettor/syringe and add one preset amount from the filtered extract in the second well of the red-marked mixing strip. Discard tip.

(6) Repeat step (5) for each subsequent sample using the designated sample wells and new pipette tip for each.

NOTE: If using a single channel pipettor or syringe, steps (5) through (8) must be performed individually and as quickly as possible.

(7) Using a pipettor/syringe, mix the contents of the mixing wells by pipetting up and down in the tips 5 times.

(8) Using a new tip for each well, transfer 100 µl from each mixing well to the corresponding antibody-coated well. Discard red mixing wells and used pipette tips.

(9) Mix the antibody-coated wells by gently sliding the microwell holder/wells back and forth on a horizontal surface for 10 to 20 seconds. Be careful not to splash solution out of wells.

(10) Set the timer and allow the antibody-coated wells to incubate for 5 minutes.

(11) After the incubation reaction is complete, shake out the contents of the antibody-coated wells.

(12) Using a wash bottle, fill each antibody-coated well with distilled/deionized water and dump out. Repeat this step 10 times.

(13) Remove all water droplets by turning wells upside down and gently tapping over a paper towel until all of the water is removed.

(14) Firmly place a new pipette tip on the pipettor/syringe and transfer 100 µl of substrate (green-capped tube) to each antibody-coated well. Discard tip.

(15) Mix the antibody-coated wells by gently sliding the microwell holder/wells back and forth on a horizontal surface for 10 to 20 seconds. Be careful not to splash solution out of wells.

(16) Set the timer and allow the antibody-coated wells to incubate for 5 minutes.

(17) After the incubation reaction is complete, firmly place a new pipette tip on the pipettor/syringe and transfer 100 µl of red stop solution (red-labeled bottle) to each antibody-coated well. Discard tip.

(18) Mix the antibody-coated wells by gently sliding the microwell holder/wells back and forth on a horizontal surface for 10 to 20 seconds. Be careful not to splash solution out of wells.

c. Interpreting Results.

Place the well strip on a white surface when determining results. Interpret the test results as follows:

(1) Equal to or less than 20 ppb.

The sample is considered equal to or less than 20 ppb when the "Sample" well is as blue or darker (blue) than the control well.

(2) Greater than 20 ppb.

The sample is considered greater than 20 ppb when the "Sample" well shows less blue color (more red color) than the control well.

7.5 REPORTING AND CERTIFYING TEST RESULTS

a. Report results on the pan ticket and inspection log as being equal to or less than a threshold (e.g., 20 ppb) or as exceeding the threshold.

b. Certify results as being equal to or less than a threshold.

c. Refer to the Certification section of the handbook for more detailed certification procedures.

7.6 CLEANING LABWARE

a. Negative Tests (# 20 ppb).

(1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used glassware, funnels, beakers, etc., wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Place materials in a garbage bag for routine trash disposal.

b. Positive Tests (> 20 ppb).

(1) Labware.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water (e.g., 100 ml bleach to 1,000 ml water). Completely submerge the used glassware, funnels, beakers, etc., and soak for at least 5 minutes. Remove items from the bleach/water solution, submerge in a dishwashing liquid/water solution, wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water in a plastic pail labeled "bleach solution". Soak disposable materials, such as used columns, cuvettes, vials, test kit components, etc., for at least 5 minutes. Pour off the liquid down the drain and place the materials in a garbage bag and discard.

7.7 WASTE DISPOSAL

a. Negative Results (# 20 ppb).

If the test result is negative (equal to or less than 20 ppb), discard the filter paper and its contents (ground material) into a plastic garbage bag for disposal. Dispose of any remaining liquid filtrate in the chemical waste container.

b. Positive Results (> 20 ppb).

If the result is positive (more than 20 ppb), the ground portion remaining in the filter paper must be decontaminated prior to disposal. After disposing of the remaining filtered extract in the chemical waste container, filter approximately 50 ml of bleach through the filter containing the ground portion and allow to drain. Discard the filter paper and its contents (ground portion) into a plastic garbage bag for disposal. The bleach rinse filtrate collected may be treated as a non-hazardous solution and disposed of by pouring down the drain.

7.8 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits

(1) Foil pouch with 24 antibody-coated wells and 24 red mixing wells.

(2) 1 yellow-labeled bottle of 20 ppb aflatoxin control solution.

(3) 1 blue-labeled bottle of enzyme conjugate solution.

(4) 1 green-labeled bottle of substrate solution.

(5) 1 spring syringe.

(6) 75 pipette tips.

b. Materials Required but not Provided:

(1) Timer (5 minute capacity).

(2) 100 µl pipettor (single or multi-channel) with tips.

(3) Microwell holder.

(4) Wash bottle.

(5) Felt tipped pens.

(6) Balance.

(7) Sample Grinder.

(8) Methanol - ACS grade or better.

(9) Deionized or Distilled Water.

(10) Blender with mixing jars.

(11) Whatman No.1 Filter Paper or Coffee Filters.

7.9 STORAGE CONDITIONS

a. Storage Conditions.

(1) Test kits should be refrigerated between 36E- 48EF.

(2) Do not freeze any of the kit components or expose reagents to temperatures greater than 95E F.

b. Precautions.

(1) Do not use kit components beyond expiration date.

(2) Do not use reagents from one kit with reagents from a different kit.

(3) Use of incubation times other than those specified may give inaccurate results.

(4) Avoid prolonged storage of kits at ambient temperatures.

CHAPTER 8

AFLATEST TEST METHOD

8.1 GENERAL INFORMATION

The Aflatest method of testing for aflatoxin uses monoclonal antibody affinity chromatography that provides for quantitative measurement of total aflatoxins (B1, B2, G1, and G2) in parts per billion (ppb) or qualitative (screening) for aflatoxin.

8.2 PREPARATION OF SOLUTIONS

Prior to beginning test procedures, prepare the solutions required for testing. The distilled/deionized water, dilute developer solution, and the HPLC grade methanol must be checked for background fluorescence with the fluorometer after properly calibrated. None of the above reagents should give a positive reading of more than 1.0 ppb.

a. Dilute Developer Solution.

NOTE: Developer Solution must be prepared fresh daily.

The concentrated developer solution should have a slight reddish brown color. (Do not use the stock solution if it is colorless.) Loss of color indicates that the stock solution has lost its potency.

Prepare dilute developer solution by adding 5 ml of Aflatest developer concentrate (Vicam Cat. # 32010) to 45 ml of distilled/ deionized water. Mix well, and label the dilute developer solution bottle showing the date and time of preparation.
DO NOT USE IT AFTER 6 HOURS HAVE ELAPSED.

If the amount of dilute developer being prepared needs to be adjusted based on the workload at individual locations, make sure that the 1 part concentrated developer to 9 parts distilled/deionized water ratio is maintained.

Label each stock bottle of concentrated developer with the date on which it was first opened. **DO NOT USE IT AFTER 30 DAYS HAVE ELAPSED.**

b. 80/20 Percent Methanol Solution.

Make up the solution by using the ratio of 8 parts HPLC grade methanol to 2 parts deionized/distilled water. Prepare the 80 percent methanol water solution by adding 800 ml methanol to 200 ml of water. Mix well. Keep the bottle tightly capped when not in use.

Label the 80 percent methanol/water solution bottle showing date of preparation. If the amount of the 80 percent methanol solution being prepared needs to be adjusted based on the workload at individual locations, make sure that the 8 parts HPLC grade methanol to 2 parts distilled/deionized water ratio is maintained.

To prepare smaller or larger amounts of solution the ratio of 8 parts methanol to 2 parts of deionized or distilled water must be maintained. For example: To prepare a solution that will provide for 5 test extractions (100 ml per test sample) mix 400 ml HPLC grade methanol to 100 ml deoinized or distilled water.

8.3 FLUOROMETER CALIBRATION

a. General.

An FGIS-approved fluorometer is used to determine the aflatoxin level. To ensure accurate results, calibrate the fluorometer prior to use each day and verify at least once an hour using the **Yellow Vial**.

Turn the fluorometer on with the On/Off switch located on the rear panel. When the fluorometer is turned on, allow it to warm up for 10 minutes before calibrating. Once the fluorometer is turned on, it may be left on until close of business for the day. If the fluorometer is turned off during the day, a 10-minute warm up is required.

After turning the fluorometer on, it will identify itself and perform a set of self-tests. If any error message appears, consult the operator's manual.

b. Calibration Procedures.

(1) Set the date, time, test delay time (60 seconds), and measurement units (ppb).

(2) Follow the prompts on the fluorometer display to calibrate the unit.

(3) When prompted to insert a calibration vial, wipe the vial with a clean cloth or paper wipe and insert it into the bottom of the well. Be sure that the vial is fully inserted and touches the bottom of the well.

(4) Enter the correct calibration value (see table below) for the high calibrator (red vial) and low calibrator (green vial).

Note: This step is applicable to the Series III and Series IV fluorometers only. Calibration values are not entered for the MF-2000 Minifluorometer.

(5) Check the calibration by testing the yellow vial.

Calibrations (in ppb) for Corn, Corn Meal, Corn/Soy Blend, Corn Germ Meal, Wheat, Sorghum, Soybeans, Flaking Corn Grits, Milled Rice, and Popcorn			
	Series III	Series IV	MF-2000
Red	150	140	*
Green	-3.0	-3.0	*
Yellow	75 ± 5	70 ± 5	66 - 74

Calibrations (in ppb) for Corn Gluten Meal and Corn Gluten Feed			
	Series III	Series IV	MF-2000
Red	110	100	*
Green	-3.0	-3.0	*
Yellow	55 ± 5	50 ± 5	66 - 74

*** Note: No values for the red and green calibrators.**

The MF-2000 does not give digital display values. Instead, a series of bar graph lights and the FGIS Aflatest overlay are used to read the yellow calibrator value. When the yellow vial is inserted, 10 bar graph lights should illuminate. This corresponds to a value between 66 - 74 ppb. Use the overlay to determine whether the value of the yellow vial is within FGIS specifications.

(6) Record the result for the Yellow Vial.

(7) If the value of the yellow calibration vial is not within FGIS specifications, repeat the calibration process (steps 2 through 4 listed above), then check the yellow vial again. If the reading for the Yellow Vial remains above or below FGIS specifcations, contact the Mycotoxin Testing Group at TSD.

(8) When the fluorometer is calibrated, place the standards back in the case and close tightly, and store away from any light source.

(9) Check the calibration of the fluorometer at least once an hour or before analyzing any test samples if more than 1 hour time has elapsed since the last test using the Yellow Vial.

c. Calibration Standards.

(1) Maintenance.

The standard solutions in the three (3) standard vials (Red, Green, and Yellow) degrade slowly in the presence of light.

Since the plastic case containing the vials passes a small amount of light, it is recommended that both case and vials be stored in a cabinet or drawer away from all light except when calibrating or checking the calibration of the fluorometer.

Maintain two (2) sets of standards (two cases) at each location. Select and identify one set as the working standard, the other as the reference standard to be used to check the working standard every 14 days.

The degradation of the working set will occur gradually over a period of time, so anticipate expiration and requisition a replacement set in advance. (A sudden change in the reading of a vial indicates instrument instability, a cracked vial, or undue exposure of the vial to light.)

When one vial of a set expires, replace the entire set. About 2 months before the expected expiration of the working set, obtain a new set of standards from Vicam Co. When received, compare fluorometer readings of the new set with those of the existing reference set. If the difference between the two sets exceeds 3 ppb for any of the colors, notify TSD.

(2) Biweekly check of working standards.

(a) Calibrate the fluorometer using the working set as described in "Calibration Procedures" (see section 8.3 b).

(b) Test the red and green vials from the working set and record the values.

(c) After calibrating the working set, remove the reference set from storage and test the 3 vials as described in section 8.3, b. The difference in readings of the two sets should not exceed the following limits:

Red	Yellow	Green
± 10 ppb	± 5 ppb	± 2 ppb

If the difference between the working and reference sets exceeds the tolerances, discard the working set. Begin using the old reference set as the working set, and use the new set as the reference set. Keep a permanent record of all calibration verification data.

8.4 SOLUTION TESTING

The distilled/deionized water, dilute developer solution, and HPLC grade methanol must be tested for background fluorescence before use. After calibrating the fluorometer perform the following:

a. Methanol.

Place 2.0 ml of HPLC grade methanol into a clean cuvette. Place the cuvette in the calibrated fluorometer. The displayed reading should be between -3.0 and +1.0. If the reading is positive and greater than 1.0, replace the methanol.

b. Water.

Dispense 2.0 ml of deionized/distilled water into a clean cuvette. Place the cuvette in the calibrated fluorometer. The digital display reading should be between -3.0 and +1.0. If the reading is positive and greater than 1.0, take action to assure a pure water supply.

c. Developer Solution.

Combine 1.0 ml of dilute developer solution and 1.0 ml of HPLC grade methanol in a clean cuvette. Place the cuvette in the calibrated fluorometer. The digital display reading should be between -3.0 and +1.0.

If the reading is positive and greater than 1.0, check each reagent separately to determine which reagent is causing the problem and replace it.

8.5 TEST PROCEDURES

a. Procedures for Testing Corn, Corn Meal, Corn/Soy Blend, Flaking Corn Grits, Milled Rice, Popcorn, Sorghum, and Soybeans.

Note: All aflatoxin tests for rice are performed on a milled rice basis. Consequently, rough rice or brown rice require milling before analysis. Mill rough rice or brown rice according to the procedures in the Rice Handbook.

(1) Extraction.

(a) Place 50 g of ground sample into blender jar.

(b) Add 5 grams of analytical, USP grade sodium chloride (NaCl) or food grade un-iodized salt.

(c) Add 100 ml of the 80/20 methanol/water extraction solution.

(d) Cover jar and blend at high speed for 1 minute.

(e) Remove the cover and pour the extract into a filter paper (Whatman 2V folded or S&S 591 24 cm pleated or equivalent) supported in a clean funnel.

(f) Collect the filtrate in a clean beaker labeled with the sample identification.

(g) After collecting approximately 25 ml of extract, carefully dispose of the filter paper and its contents.

(h) Pipette 5 ml of filtered extract into a clean beaker.

(i) Add 10 ml of distilled/deionized water and mix thoroughly.

(j) Filter the diluted extract through a glass microfibre filter (Vicam Cat. # 31955) supported by a small, clean funnel. Fold the glass microfibre filter gently without making a sharp crease to avoid breaking the glass microfibre filter.

(k) Immediately proceed with the Aflatest Affinity Column procedure.

Note: If this diluted filtrate turns cloudy, refilter using a new glass microfibre filter before proceeding with the analysis.

(2) Affinity Column.

(a) Prepare an Aflatest-P affinity column for use by removing both end caps and gently shaking the buffer solution from the top of the column.

(b) Using an Eppendorf pipette, or equivalent, add 1.0 ml of the filtered dilute extract to the top of the Aflatest column.

(c) Attach the column to the washing device (either a syringe barrel or an air pumping station) and pass the filtered extract through the column using a steady positive pressure. Maintain a flow rate of approximately 1 drop per second.

Note: Sample analysis using these procedures can be greatly simplified by the use of a small aquarium air pump to provide the needed air pressures for loading, filtering, and washing the various extracts.

(d) After the extract has completely passed through the Aflatest column, add 1 ml of deionized or distilled water to the column and again apply a steady positive pressure to pass the wash water through the column. (If a syringe barrel rather than the pumping station is used, detach the column and pipette 1 ml of deionized or distilled water into the column headspace.) Reattach the column to the syringe barrel and apply pressure to pass the water through the column.

(e) Repeat the water wash in step (d) above.

(f) After the second wash has passed through the column, place a clean cuvette under the outlet of the column. Only 12 x 75 mm borosilicate glass tubes should be used for cuvettes (Vicam Cat. # 34000 or equivalent). Use care when handling the cuvette to keep the optical surface clean and free of lint, fingerprints, etc.

(g) Dispense 1.0 ml of HPLC grade methanol into the column. If a syringe barrel rather than the pumping station is used, detach the column, pipette 1 ml of methanol directly into the column headspace, and replace the column.

(h) Apply a steady pressure to elute/pass the methanol through the column and collect all of the methanol eluate in the cuvette. Maintain pressure to collect the methanol at a rate of approximately 1 drop per second.

(i) Add 1.0 ml of dilute Aflatest Developer Solution directly to the sample eluate solution in the cuvette and mix well (about 5 seconds).

(j) **Immediately** place the cuvette in a calibrated fluorometer.

(3) Reading, Recording, and Certifying Test Results.

(a) Record the digital readout (Series III and IV) or corresponding bar graph value (MF-2000) as total ppb.

(b) Report all results on the pan ticket and the inspection log to the nearest whole ppb.

(c) Sample results over 300 ppb are reported as >300 ppb unless a supplemental analysis is performed.

(d) Refer to the Certification section of the handbook for more detailed certification procedures.

(4) Supplemental Analysis.

To determine and report an aflatoxin level higher than 300 ppb, the filtered test sample extract must be diluted so that a value between 5 ppb and 300 ppb is obtained. The final aflatoxin concentration is calculated by multiplying the results obtained with the diluted extract by the dilution factor.

(a) Using an Eppendorf pipette, add 0.5 ml (instead of 1.0 ml) of the filtered diluted extract to the top of the Aflatest column headspace. (See section 8.5 a (2) (b).)

(b) Analyze the filtered extract as a normal sample.

(c) Multiply the analytical results obtained by 2 to obtain the actual aflatoxin concentration. For example, if 240 ppb was the sample value obtained using the diluted test sample procedure, the actual concentration in the original sample was 480 ppb.

Example:	Diluted test sample extract result	240 ppb
	Dilution factor	x 2
	Actual aflatoxin concentration	480 ppb

Note: Laboratories may dilute samples as a first step if levels typically observed exceed 300 ppb and the applicant requests certified results above the range of the test kit.

b. Procedures for Testing Corn Germ Meal and Wheat.

(1) Extraction.

(a) Place 50 g of ground sample into blender jar.

(b) Add 10 grams of analytical, USP grade sodium chloride (NaCl) or food grade un-iodized salt.

(c) Add 200 ml of the 80/20 methanol/water extraction solution.

(d) Cover jar and blend at high speed for 1 minute.

(e) Remove the cover and pour the extract into a filter paper (Whatman 2V folded or S&S 591 24 cm pleated or equivalent) supported in a clean funnel.

(f) Collect the filtrate in a clean beaker labeled with the sample identification.

(g) After collecting approximately 25 ml of extract, carefully dispose of the filter paper and its contents.

(h) Pipette 5 ml of filtered extract into a clean beaker.

Note: If the solution filtration is slow (i.e., more than two minutes are required to collect 5 ml of filtrate), withdraw 5.0 ml of the clearest liquid from the top of the material held in the funnel (see step (e) above) and transfer it to a clean container.

(i) Add 10 ml of distilled/deionized water and mix thoroughly.

(j) Filter the diluted extract through a glass microfibre filter (Vicam Cat. # 31955) supported by a small, clean funnel. Fold the glass microfibre filter gently without making a sharp crease to avoid breaking the glass microfibre filter.

(k) Immediately proceed with the Aflatest Affinity Column procedure.

Note: If this diluted filtrate turns cloudy, refilter using a new glass microfibre filter before proceeding with the analysis.

(2) Affinity Column.

(a) Prepare an Aflatest-P affinity column for use by removing both end caps and gently shaking the buffer solution from the top of the column.

(b) Using an Eppendorf pipette, add 1.0 ml of the filtered dilute extract to the top of the Aflatest column.

(c) Attach the column to the washing device (either a syringe barrel or an air pumping station) and pass the filtered extract through the column using a steady positive pressure. Maintain a flow rate of approximately 1 drop per second.

Note: Sample analysis using these procedures can be greatly simplified by the use of a small aquarium air pump to provide the needed air pressures for loading, filtering, and washing the various extracts.

(d) Using an Eppendorf pipette, add 1.0 ml of the filtered dilute extract to the top of the Aflatest column.

(e) Attach the column to the washing device (either a syringe barrel or an air pumping station) and pass the filtered extract through the column using a steady positive pressure. Maintain a flow rate of approximately 1 drop per second.

(f) After the extract has completely passed through the Aflatest column, add 1 ml of deionized or distilled water to the column and again apply a steady positive pressure to pass the wash water through the column. (If a syringe barrel rather than the pumping station is used, detach the column and pipette 1 ml of deionized or distilled water into the column headspace.) Reattach the column to the syringe barrel and apply pressure to pass the water through the column.

(g) Repeat the water wash in step (f) listed above.

(h) After the second wash has passed through the column, place a clean cuvette under the outlet of the column. Only 12 x 75 mm borosilicate glass tubes should be used for cuvettes (Vicam Cat. # 34000 or equivalent). Use care when handling the cuvette to keep the optical surface clean and free of lint, fingerprints, etc.

(i) Dispense 1.0 ml of HPLC grade methanol into the column. If a syringe barrel rather than the pumping station is used, detach the column, pipette 1 ml of methanol directly into the column headspace, and replace the column.

(j) Apply a steady pressure to elute/pass the methanol through the column and collect all of the methanol eluate in the cuvette. Maintain pressure to collect the methanol at a rate of approximately 1 drop per second.

(k) Add 1.0 ml of dilute Aflatest Developer Solution directly to the sample eluate solution in the cuvette and mix well (about 5 seconds).

(l) **Immediately** place the cuvette in a calibrated fluorometer.

(3) Reading, Recording, and Certifying Test Results.

(a) Record the digital readout (Series III and IV) or corresponding bar graph value (MF-2000) as total ppb.

(b) Report all results on the pan ticket and the inspection log to the nearest whole ppb.

(c) Sample results over 300 ppb are reported as >300 ppb unless a supplemental analysis is performed.

(d) Refer to the Certification section of the handbook for more detailed certification procedures.

c. Procedures for Testing Corn Gluten Meal and Corn Gluten Feed.

(1) Extraction.

(a) Place 50 g of ground sample into blender jar.

(b) Add 5 grams of analytical, USP grade sodium chloride (NaCl) or food grade un-iodized salt.

(c) Add 250 ml of 60 percent methanol/40 percent water extraction solution to blender jar. The 60/40 percent methanol/water solution is prepared by mixing 600 ml HPLC grade methanol with 400 ml distilled/deionized water.

(d) Cover jar and blend at high speed for 1 minute.

(e) Remove the cover and pour the extract into a filter paper (Whatman 2V folded or S&S 591 24 cm pleated or equivalent) supported in a clean funnel.

(f) Collect the filtrate in a clean beaker labeled with the sample identification.

(g) After collecting approximately 25 ml of extract, carefully dispose of the filter paper and its contents.

(h) Pipette 10 ml of filtered extract into a clean beaker.

(i) Add 20 ml of distilled/deionized water and mix thoroughly.

(j) Filter the diluted extract through a glass microfibre filter (Vicam Cat. # 31955) supported by a small, clean funnel. Fold the glass microfibre filter gently without making a sharp crease to avoid breaking the glass microfibre filter.

(k) Load 6-8 ml of the filtrate from step (j) above into a 10 ml plastic syringe barrel fitted with 0.22 micron nylon syringe disk filter (Fisher Scientific Corporation CAMEO II Cat. No. DDN 02T2550, Gelman Cat. No. 09-730-191, or Corning Cat. No. 09-754-22).

(l) Apply enough air pressure to syringe barrel to produce a flow of approximately 1 drop per second through disk filter and collect a minimum of 5 ml of filtrate in a clean test tube. Discard filter disk.

(2) Affinity Column.

(a) Prepare an Aflatest-P affinity column for use by removing both end caps and gently shaking the buffer solution from the top of the column.

(b) Using a 1.0 ml Eppendorf pipette, load 4.0 ml of refiltered extract from step (l) above into the barrel of a 10 ml glass syringe to which an Aflatest-P column is attached.

(c) Apply pressure so that the extract passes through the column at 1 to 2 drops per second. Remove syringe barrel from column. Fill column with distilled water. Reattach syringe barrel to column.

(d) Fill syringe barrel with 10 ml of distilled/deionized water and pass through column at a flow rate of approximately 2 drops per second. Allow all of wash water to pass through column.

(e) Repeat column wash with another 10 ml of deionized/distilled water.

(f) Elute aflatoxin from Aflatest-P column with 1 ml HPLC grade methanol and collect sample eluate solution in glass cuvette.

(g) Add 1 ml of fresh, dilute Aflatest developer solution directly to the eluate in cuvette and mix well.

(h) **Immediately** place the cuvette in a calibrated fluorometer.

(3) Reading, Recording and Certifying Test Results.

(a) Record the digital readout (Series III and IV) as total ppb. **To determine the aflatoxin concentration using the MF-2000 fluorometer, read the corresponding bar graph value and multiply by 0.73 for actual ppb.**

(b) Report all results on the pan ticket and the inspection log to the nearest whole ppb.

(c) Sample results over 300 ppb are reported as >300 ppb.

(d) Refer to the Certification section of the handbook for more detailed certification procedures.

NOTE: Rinse both glass and plastic syringe barrels with approximately 10 ml of distilled/deionized water each before analyzing next sample.

8.6 CLEANING LABWARE

a. Negative Tests (# 20 ppb).

(1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used glassware, funnels, beakers, etc., wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Place materials in a garbage bag for routine trash disposal.

b. Positive Tests (> 20 ppb).

(1) Labware.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water (e.g., 100 ml bleach to 1,000 ml water). Completely submerge the used glassware, funnels, beakers, etc., and soak for at least 5 minutes. Remove items from the bleach/water solution, submerge in a dishwashing liquid/water solution, wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water in a plastic pail labeled "bleach solution." Soak disposable materials, such as used columns, cuvettes, vials, test kit components, etc., for at least 5 minutes. Pour off the liquid down the drain and place the materials in a garbage bag and discard.

8.7 WASTE DISPOSAL

a. Negative Results (# 20 ppb).

If the test result is negative (equal to or less than 20 ppb), discard the filter paper and its contents (ground material) into a plastic garbage bag for disposal. Dispose of any remaining liquid filtrate in the chemical waste container.

b. Positive Results (> 20 ppb).

If the result is positive (more than 20 ppb), the ground portion remaining in the filter paper must be decontaminated prior to disposal. After disposing of the remaining filtered extract in the chemical waste container, filter approximately 50 ml of bleach through the filter containing the ground portion and allow to drain. Discard the filter paper and its contents (ground portion) into a plastic garbage bag for disposal. The bleach rinse filtrate collected may be treated as a non-hazardous solution and disposed of by pouring down the drain.

8.8 EQUIPMENT and SUPPLIES

a. Fluorometer - Romer model RL-100, Vicam Series III and IV, or Vicam model MF-2000.

b. Fluorometer calibration standards. (Vicam # 33030)

c. Cuvette Rack. (Vicam # 21010)

d. Pump assembly stand, double. (Vicam # 21030)

e. Syringe, glass 10 ml. (Vicam # 34010)

f. Syringe hand pump with coupling. (Vicam #36030)

g. Automatic pipettor (1 ml capacity for methanol). (Vicam #20501)

h. Automatic pipettor (1 ml capacity for developer). (Vicam #20600)

i. Graduated cylinders - 25 ml, 100 ml, and 250 ml capacity.

j. Aflatest-P columns. (Vicam # 12022)

k. Cuvettes, disposable 12 x 75 mm borosilicate glass tube. (Vicam # 34000)

l. Disposable beakers. (Vicam # 36010)

m. Glass microfibre filter paper -Whatman 934-AH. (Vicam # 31955)

n. Small plastic funnels.

o. Wash bottles or spray bottles.

p. Box of Kim Wipes (small size sheets).

q. HPLC grade methanol.

r. Aflatest developer solution. (Vicam # 32010)

s. Balance.

t. Sample Grinder.

u. Distilled/deionized water.

v. Aflatest developer solution. (Vicam #32010)

w. USP grade sodium chloride (NaCl) or food grade un-iodized salt.

8.9 STORAGE CONDITIONS

a. Affinity Columns - Store at room temperature (64º to 86º F).

b. Calibration Vials - Store in a cabinet or drawer away from all light, except when in use.

c. Developer Concentrate - Store in a tightly closed bottle in a cool, dry, well ventilated area and away from sunlight, combustible materials, and incompatible materials.

CHAPTER 9

FLUOROQUANT TEST METHOD

9.1 GENERAL INFORMATION

The Romer Fluoroquant aflatoxin test method uses fluorescence technology to quantitatively measure total aflatoxins (B1, B2, G1, and G2) in parts per billion (ppb).

9.2 PREPARATION OF SOLUTIONS

a. Developer Solution.

Prepare the working developer reagent by adding 50 ml of deionized or distilled water to the bottle supplied with the repipettor and then adding the contents of one ampule of developer concentrate.

To insure complete and accurate transfer, rinse the ampule three times with the working developer solution, each time returning the rinse to the bottle. Swirl to mix contents.

There is room in the repipettor bottle to make a larger amount of developer by adding the contents of 2 ampules to 100 ml of water.

Initially prime the repipettor in a waste tube to remove any air bubbles. When using the repipettor, pull up the plunger all the way to the stop, then push all the way back down to insure accurate delivery volume. Before each use, prime the repipettor slightly to remove any air that may form at the tip. Place the cap on the end of the repipettor tip after each use.

NOTE: The developer working reagent must be made fresh every 8 hours.

b. 80 Percent Methanol Solution **(for corn, corn meal, rice, popcorn, sorghum, and wheat)**.

(1) Using a graduated cylinder, measure 800 ml of methanol (HPLC grade) and place it into a clean carboy with spigot.

(2) Add 200 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed.

(3) Label the container stating the mixture (80 percent methanol and 20 percent water), date of preparation, and initials of technician who prepared the solution.

(4) Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 8 parts methanol to 2 parts of deionized or distilled water.

c. 90 Percent Acetonitrile Solution **(for soybeans and corn/soy blend)**.

(1) Using a graduated cylinder, measure 900 ml of acetonitrile and place it into a clean carboy with spigot.

(2) Add 100 ml deionized or distilled water.

(3) Label the container stating the mixture (90 percent acetonitrile and 10 percent water), date of preparation, and initials of technician who prepared the solution.

(4) Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 9 parts acetonitrile to 1 part of deionized or distilled water.

9.3 FLUOROMETER CALIBRATION

a. Turn the power on and respond as indicated (no warm-up period is required).

b. When first turned on, the fluorometer will go through a series of self-tests. After the self-tests are completed, the screen will allow you to change the date and time, or continue. You will then come to the "METHOD" screen. This screen allows you to choose a particular method, print a list of methods, or set up a new method. If the fluorometer has not yet been programmed with the appropriate method, use the following directions.

(1) Press the "Set Up" key.

(2) Enter the method number according to the sample matrix being tested and press "Enter".

(3) Enter the delay time as 40 seconds.

(4) Select the measurement as "ppb".

(5) Select the results as "decimals".

(6) Enter the **High Calibrator** value according to the calibration card provided.

(7) Enter the **Low Calibrator** value as stated and continue with the procedure.

c. Press the "Select" key.

d. Enter the method number according to the sample matrix being tested and press "Enter".

e. After choosing the method desired, place the high and low calibrator ampules in the fluorometer when specified.

f. For the first sample, insert the control into the fluorometer and read the result. The control range will read as stated on the control ampule bottle. If the reading is not in this range, try recalibrating the machine. If the control value is still out of range, contact the Technical Services Department at Romer Labs Inc. at 1-800-769-1380.

9.4 EXTRACTION PROCEDURES

a. Transfer 50 grams of ground sample into an extraction mixing jar.

b. Add 100 ml of the extraction solvent.
(methanol/ water for corn, corn meal, rice, popcorn, sorghum, and wheat)
(acetonitrile/water for soybeans and corn/soy blend)

c. Cover the extraction jar and blend on high speed for 1 minute.

d. Remove the cover and funnel the extract through a Whatman No.1 filter or a coffee filter into a sample jar labeled with the sample identification.

e. After collecting the filtrate, remove the funnel, filter, and ground material and place over an empty collection container (e.g., disposable plastic beaker).

9.5 TEST PROCEDURES

a. Purification of Corn, Corn Meal, Rice, Popcorn, Sorghum, and Wheat.

(1) Make sure the clear plastic tip is pushed firmly onto the bottom of the UniSep 2001 column. This prevents any solution from passing through the column prematurely.

(2) Place 1 ml of the extract in the top of the column and discard the pipette tip.

(3) Add 1 ml of the diluent and discard the pipette tip.

(4) Place the blue cap on top of the column and mix thoroughly by hand, shaking vigorously for 5 seconds.

(5) Uncap the top and bottom of the column and place the column in a 12 x 75 mm cuvette. Insert the syringe barrel and stopper into the top of the column.

(6) Slowly **(30-40 seconds)** push the extract through the column until air comes out of the bottom.

NOTE: It is critical to push the solution completely through the column in at least a 30-40 second time-frame indicated to insure a complete extraction solution purification.

(7) Transfer 0.5 ml of each purified sample extract to a clean 12 x 75 mm cuvette and cap.

b. Purification of Soybeans and Corn/Soy Blend.

(1) Make sure the clear plastic tip is pushed firmly onto the bottom of the UniSep 2001 column. This prevents any solution from passing through the column prematurely.

(2) Place 2 ml of the extract in the top of the column and discard the pipette tip.

(3) Place the blue cap on top of the column and mix thoroughly by hand, shaking vigorously for 5 seconds.

(4) Uncap the top and bottom of the column and place the column in a 12 x 75 mm cuvette. Insert the syringe barrel and stopper into the top of the column.

(5) Slowly **(30-40 seconds)** push the extract through the column until air comes out of the bottom.

NOTE: It is critical to push the solution completely through the column in at least a 30-40 second time-frame indicated to insure a complete extraction solution purification.

(6) Transfer 250 µl (0.25 ml) of each purified sample extract to a clean 12 x 75 mm cuvette.

(7) Add 250 µl of methanol to the cuvette and cap.

c. Derivatization and Fluorometric Reading.

(1) Immediately add 1 ml of the developer working reagent to each purified sample.

(2) Recap the tube and vortex for 5 seconds.

(3) Wipe the cuvette with lint-free paper and place in the fluorometer for a reading.

(4) After a 40-second delay, the result will appear on the fluorometer screen and a record will be printed out.

NOTE: Once the developer reagent is added, the sample must be mixed, and the sample cuvette must be placed in the fluorometer quickly. Samples must be derivatized one sample at a time and then read before proceeding to the next sample.

9.6 REPORTING AND CERTIFYING TEST RESULTS

a. Record the digital readout as ppb total aflatoxins in the sample.

b. Report all results on the pan ticket and the inspection log to the nearest whole ppb.

c. Sample results over 300 ppb are reported as >300 ppb unless a supplemental analysis is performed.

d. Refer to the Certification section of the handbook for more detailed certification procedures.

9.7 SYSTEM CHECK

a. Positive Control Option.

The test kit contains a positive control standard that may be used as a check on method technique and overall system performance.

b. System Check Procedures.

(1) Make sure the clear plastic tip is pushed firmly onto the bottom of the UniSep2001 column.

(2) Place 1 ml of **80/20 methanol/water solution** in the top of the column and discard the pipette tip.

Note: Do not use the 90/10 acetonitrile/water solution in this procedure.

(3) Add 1 ml of Positive Control Standard and discard the pipette tip.

(4) Place the blue cap on top of the column and mix thoroughly by hand shaking vigorously for 5 seconds.

(5) Uncap the top and bottom of the column and place the column in a 12 x 75 mm cuvette. Insert the syringe barrel and stopper into the top of the column.

(6) Slowly **(30-40 seconds)** push the extract through the column until air comes out the bottom.

(7) Transfer 0.5 ml of the purified positive control solution to a clean 12 x 75 mm cuvette and cap.

(8) Immediately add 1 ml of developer and working reagent to the positive control standard.

(9) Recap the tube and vortex for 5 seconds.

(10) Wipe the cuvette with lint-free paper and place in the fluorometer for a reading.

(11) After a 40-second delay, the result will appear on the fluorometer screen and a record will be printed out. The value received should fall within the range listed on the label of the bottle.

NOTE: Be sure to use the corn calibration factors to test the control standard.

9.8 SUPPLEMENTAL ANALYSIS

a. Diluting the Sample Extract.

If quantitative results are above the test method's conformance limit, test results are reported as exceeding the limit. To determine and report an aflatoxin level higher than 300 ppb, the sample extract must be diluted so that a value between 5 and 300 ppb is obtained. The final aflatoxin concentration is calculated by multiplying the results with the diluted extract by the dilution factor.

b. Example.

If the original analysis reported the aflatoxin value at 700 ppb, the sample extract would be diluted using the following procedures in order to obtain a true value.

(1) Dilute 5 ml of the original extract with 10 ml of the extraction solvent mixture **(methanol/ water for corn, corn meal, rice, popcorn, sorghum, and wheat**, **acetonitrile/water for soybeans and corn/soy blend).** The total volume is 15 ml. This is a 1 to 3 dilution (compares volume in the beginning with the total volume in the end).

(2) Multiply the analytical results obtained by 3 to obtain the actual aflatoxin concentration. For example, if 240 ppb was the original value obtained with the diluted extract, the actual concentration in the original sample was 720 ppb.

$$\text{True Aflatoxin Value} = \frac{\text{Total Volume}}{\text{Initial Extract Volume}} \times \text{Aflatoxin Result}$$

True Aflatoxin Value = (15) 5) x 240 ppb
= 3 x 240 ppb = 720 ppb

9.9 CLEANING LABWARE

a. Negative Tests (# 20 ppb).

(1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used glassware, funnels, beakers, etc., wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Place materials in a garbage bag for routine trash disposal.

b. Positive Tests (> 20 ppb).

(1) Labware.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water (e.g., 100 ml bleach to 1,000 ml water). Completely submerge the used glassware, funnels, beakers, etc., and soak for at least 5 minutes. Remove items from the bleach/water solution, submerge in a dishwashing liquid/water solution, wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water in a plastic pail labeled "bleach solution". Soak disposable materials, such as used columns, cuvettes, vials, test kit components, etc., for at least 5 minutes. Pour off the liquid down the drain and place the materials in a garbage bag and discard.

9.10 WASTE DISPOSAL

a. Negative Results (# 20 ppb).

If the test result is negative (equal to or less than 20 ppb), discard the filter paper and its contents (ground material) into a plastic garbage bag for disposal. Dispose of any remaining liquid filtrate in the chemical waste container.

b. Positive Results (> 20 ppb).

If the result is positive (more than 20 ppb), the ground portion remaining in the filter paper must be decontaminated prior to disposal. After disposing of the remaining filtered extract in the chemical waste container, filter approximately 50 ml of bleach through the filter containing the ground portion and allow to drain. Discard the filter paper and its contents (ground portion) into a plastic garbage bag for disposal. The bleach rinse filtrate collected may be treated as a non-hazardous solution and disposed of by pouring down the drain.

9.11 EQUIPMENT AND SUPPLIES

a. Blender with ½ pint jars.

b. Syringe with rubber stopper.

c. Cuvette rack.

e. Pipettor and tips - 200 to 1000 µl (.20 to 1 ml) adjustable.

f. Vortex Mixer.

g. Fluorometer (Vicam Series III or IV, or Romer RL100) and printer.

h. 100 ml graduated cylinder.

i. Funnel.

j. Timer.

k. Whatman No.1 Filter Paper or Coffee Filters.

l. Glass cuvettes (12 x 75 mm).

m. Empty bottles for Developer Working Reagent and Working Diluent.

n. Repipette Dispenser (1ml), Labindustries Model LS830X3 or equivalent.

o. Sample grinder.

p. Balance.

q. HPLC grade Methanol (**for extraction solvent for corn, corn meal, rice, popcorn, sorghum, and wheat).**

r. Acetonitrile **(for extraction solvent for soybeans and corn/soy blend).**

s. Deionized or Distilled Water.

t. UniSep 2001 Fluoroquant "A" columns.

u. Developer Concentrate.

v. Diluent (**for corn, corn meal, rice, popcorn, sorghum, and wheat).**

w. High, Low, and Control calibrator ampules.

x. Positive Control Standard.

9.12 STORAGE CONDITIONS

a. UniSep 2001 columns - Room temperature in a sealed container.

b. Developer Concentrate - shipped in an amber bottle. Store in a tightly closed container in a cool, dry, well ventilated area away from direct sunlight, combustible materials, and incompatible substances.

c. Calibrators - Room temperature.

d. Diluent - In a cool place away from heat source.

e. Positive Control Standard - Refrigerated at 23º to 32º F.

CHAPTER 10

VERATOX-AST TEST KIT

10.1 GENERAL INFORMATION

The Veratox AST test is a quick diagnostic tool to predict the presence of aflatoxin in corn and other commodities. The kit uses an enzyme-linked immunosorbent assay (ELISA) technique to obtain quantitative results from absorbance readings at 650 nm when sample readings are compared to a 20 ppb control and a pre-generated standard curve (0 to 400 ppb).

10.2 PREPARATION OF EXTRACTION SOLUTION

The extraction solvent used in the Veratox test method is a methanol/water (distilled or deionized) mixture consisting of 70 percent methanol (ACS grade or better) and 30 percent water.

a. Using a graduated cylinder, measure 700 ml of methanol and place it into a clean carboy with spigot.

b. Add 300 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed.

c. Label the container stating the mixture (70 percent methanol and 30 percent water), date of preparation, and initials of technician who prepared the solution.

d. Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 7 parts methanol to 3 parts of deionized or distilled water.

10.3 EXTRACTION PROCEDURES

a. Place a sheet of filter paper (Whatman 2V folded or S&S 24 cm pleated or equivalent) into a clean funnel mounted over a 25 X 200 mm (diameter x length) test tube or a collection beaker.

b. Label the collection container with the sample identification.

c. Place the 50-gram portion of the ground sample into the blender container.

d. Pour in 250 ml of the 70/30 percent methanol/water solution and securely close the blender top.

e. Blend for exactly two minutes at high speed.

f. Pour the resultant mixture from the blender into the funnel containing the filter paper and collect approximately 25 ml of extract.

10.4 TEST PROCEDURES

a. Preparation of Solutions.

(1) Place 3 ml of substrate (light green labeled bottle) solution into a clean, labeled reagent boat. Cover boat to protect solution from dust and light.

NOTE: Do not return any substrate solution to the original bottle once it has been removed.

(2) Place 3 ml of Red Stop (red labeled bottle) solution into a clean reagent boat. Cover boat to protect solution from dust and light.

b. Sample Analysis.

Do not use reagents or microwells from one kit serial number with reagents/wells from a different serial number. Reagent boats may be rinsed and reused.

(1) Open foil bag and remove 3 red-marked mixing wells for each sample to be tested (maximum of 4 samples or 12 wells). Place them in the microwell holder, and mark the left end of each strip with a "1."

Do not run more than four samples at one time.

(2) Remove 3 antibody wells for each sample to be tested (maximum of 4 samples or 12 wells). Place them in the microwell holder, and mark the left end of each strip with a "1."

(3) Reseal bag by folding over and tightly closing with a suitable fastener (large paper clip, tape, or suitable dust and light protectant).

(4) Place 100 µl of conjugate (blue-labeled bottle) into each mixing well using a 100 µl pipettor with a new tip. Prime the pipette tip first before dispensing the 100 µl. Discard the pipette tip.

NOTE: "Prime the pipette tip" is accomplished by drawing liquid up into the tip and dispensing it back into the bottle once or twice.

(5) Place 100 µl of control (yellow-labeled bottle) into the first mixing well labeled "1." Prime the tip before dispensing. If testing more than one sample, also place 100 µl of control into mixing well #4 for the second sample, mixing well #7 for the third sample, and mixing well #10 for the fourth sample. Discard the pipette tip.

(6) Place 100 µl of sample each in mixing wells #2 and #3. Prime the tip first before dispensing. Discard the tip. Subsequent samples should be placed in wells #5 and #6, then #8 and #9, and then #11 and #12.

See the diagram below for an example of the procedure.

	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12
mixing wells	O	O	O	O	O	O	O	O	O	O	O	O
	C	S1	S1	C	S2	S2	C	S3	S3	C	S4	S4

"W" = well number (e.g., #1 through #12)
"C" = control
"S1, S2, S3, & S4" = sample numbers

(7) Using the 12 channel pipettor and the overfill method (see note below), mix the contents of the mixing wells by pipetting up and down in the tips 5 times.

NOTE: The "overfill method" is performed by drawing greater than 100 µl into the pipette tips by pressing the pipettor to the second stop before placing tips into the solution. Place tips into the liquid and release the plunger slowly and completely.

(8) Transfer 100 µl to the antibody coated wells (the unmarked, clear wells). To dispense only 100 µl, press plunger to the first stop.

(9) Mix in the antibody coated wells by gently sliding the microwell holder back and forth on a horizontal surface for 15 seconds. Be careful not to allow solution to splash out of wells.

(10) Immediately following mixing, incubate for 5 minutes. Discard all mixing (red marked) wells and tips.

(11) With a wash bottle containing deionized/distilled water, fill each antibody well and dump the contents into a waste receptacle. Repeat this step five times.

(12) Turn microwell holder, with wells in it, upside down on a paper towel and tap gently until water is removed from the wells.

(13) Using the 12 channel pipettor and the overfill method, place 100 µl of substrate into each well.

(14) Mix gently by sliding the microwell holder back and forth for 15 seconds on a horizontal surface for 15 seconds. Be careful not to allow solution to splash out of wells.

(15) Immediately following mixing, incubate for 5 minutes.

(16) Discharge the remaining substrate in the pipette tips by plunging once or twice without drawing any additional liquid up into the tips. Save these tips for the next step.

(17) Using the 12 channel pipettor and the overfill method, add 100 µl of the red stop solution (red labeled bottle) into each well.

(18) Mix gently by sliding the microwell holder back and forth for 15 seconds. Again be careful not to lose any solution from the wells. Visually check the appearance of the wells. Discard all pipette tips.

(19) Read in a Bio Tek EL 301microwell reader using a 650 nm filter within 5 minutes of the addition of the red stop solution.

c. Reading Results with Microwell Reader.

(1) Turn on the power to reader at the beginning of the test procedure to allow the electronics to stabilize. Make sure that the reader is properly attached to the computer.

(2) Turn on the computer and insert the VERATOX-AST software disc into the drive slot.

(3) Start the VERATOX program and select option "A" -"RUN AST."

(4) Check the kit identification and the standard curve values with the Standard Curve Program Calculated Points that came with the test kit. Edit standard or kit lot numbers as necessary.

(5) Press the "Enter" key, then press the "R" key to ready the computer to receive data from the microwell reader.

(6) Calibrate the microwell reader by following the instructions which appear on the LCD window of the reader.

(a) Remove sample carrier and press the "Enter" key.

(b) Place the filter holder in the W2 position and press "Enter." The instrument will calibrate on the W2 filter.

(c) Move the filter holder to the W1 position and press "Enter." The instrument will calibrate on the W1 filter.

NOTE: The Micro-well reader used in the official aflatoxin testing service is designed to do several testing functions. Each function requires specific set-up parameters. The required parameters for aflatoxin testing are: AF1 set up L S P, 12S, ABSORB, N Y N.≅ To ensure that the Micro-well reader is properly set for aflatoxin testing, periodically check the display set-up as follows:

(d) Press the Display Set up. Display should read F1 setup L S P 12S ABSORB N Y N.

If the display reads differently, contact the Neogen Corporation representative for instruction. Otherwise, press the Display Set up again. This will return the instrument to normal operational mode.

(e) Press the "Clear" key, then the "Blank" key. This will blank the instrument on air and it is now ready to measure absorbance.

(f) Place the wells into the reader's sample holder. Make sure that the well marked "1" is in the far left position in the holder.

(g) Move the holder to the left so that the first well is under the reader and press the "Read" key. Repeat this process until all wells are read.

(h) Follow the instructions as requested by the software. Values displayed on the computer screen will be the mean of the duplicate measurements.

d. Troubleshooting.

The Veratox AST quantitative test requires two duplicate portions of a sample to be run in addition to a control portion. The mean OD reading of the duplicate portions is compared with the calculated OD points in the standard curve program to obtain a result in ppb. If the coefficient of variance (CV) between the OD values of the test portions is not within the required 15 percent range of difference, a "CV overrange≅ error will be displayed as the test result. Typically, a "CV overrange≅ message indicates that the test results are not valid and that another portion must be tested.

If the OD readings of the duplicate portions both indicate that the aflatoxin level is less than 300 ppb and a "CV overrange≅ message appears, then an additional sample portion must be tested until the OD values of duplicate portions are within 15 percent of the mean OD and the "CV overrange≅ message is eliminated. If the OD readings for the duplicate portions indicate that one of the portions exceeds 300 ppb and the other portion is less than 300 ppb and a "CV overrange≅ message is displayed, the sample must be retested (full strength or diluted portion) until both of the OD readings are within the 15 percent range.

If the OD readings for the duplicate portions both indicate that the aflatoxin content exceeds 300 ppb but a "CV overrange≅ message appears, then:

(1) an additional sample portion (diluted) must be tested until the OD values of duplicate portions are within 15 percent of the mean OD,

or

(2) official personnel may stop the testing if the applicant requests only a certification statement that aflatoxin exceeds 300 ppb. In this instance, official personnel will certificate the aflatoxin testing results as a "Aflatoxin exceeds 300 ppb.≅

10.5 REPORTING AND CERTIFYING TEST RESULTS

a. Report all results on the pan ticket and the inspection log to the nearest whole ppb.

b. Sample results over 300 ppb are reported as >300 ppb unless a supplemental analysis is performed.

c. Refer to the Certification section of the handbook for more detailed certification procedures.

10.6 SUPPLEMENTAL ANALYSIS

a. Diluting the Sample Extract.

If quantitative results are above the test method's conformance limit, test results are reported as exceeding the limit. To determine and report an aflatoxin level higher than 300 ppb, the sample extract must be diluted so that a value between 5 and 300 ppb is obtained.

The final aflatoxin concentration is calculated by multiplying the results with the diluted extract by the dilution factor.

b. Example.

If the original analysis reported the aflatoxin value at 700 ppb, the sample extract would be diluted using the following procedures in order to obtain a true value.

(1) Dilute 5 ml of the original extract with 10 ml of the extraction solvent mixture. The total volume is 15 ml. This is a 1 to 3 dilution (compares volume in the beginning with the total volume in the end).

(2) Multiply the analytical results obtained by 3 to obtain the actual aflatoxin concentration. For example, if 240 ppb was the original value obtained with the diluted extract, the actual concentration in the original sample was 720 ppb.

$$\text{True Aflatoxin Value} = \frac{\text{Total Volume}}{\text{Initial Extract Volume}} \times \text{Aflatoxin Result}$$

True Aflatoxin Value = (15) 5) x 240 ppb
= 3 x 240 ppb = 720 ppb

10.7 CLEANING LABWARE

a. Negative Tests (# 20 ppb).

(1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used glassware, funnels, beakers, etc., wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Place materials in a garbage bag for routine trash disposal.

b. Positive Tests (> 20 ppb).

(1) Labware.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water (e.g., 100 ml bleach to 1,000 ml water). Completely submerge the used glassware, funnels, beakers, etc., and soak for at least 5 minutes. Remove items from the bleach/water solution, submerge in a dishwashing liquid/water solution, wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water in a plastic pail labeled "bleach solution." Soak disposable materials, such as used columns, cuvettes, vials, test kit components, etc., for at least 5 minutes. Pour off the liquid down the drain and place the materials in a garbage bag and discard.

10.8 WASTE DISPOSAL

a. Negative Results (# 20 ppb).

If the test result is negative (equal to or less than 20 ppb), discard the filter paper and its contents (ground material) into a plastic garbage bag for disposal. Dispose of any remaining liquid filtrate in the chemical waste container.

b. Positive Results (> 20 ppb).

If the result is positive (more than 20 ppb), the ground portion remaining in the filter paper must be decontaminated prior to disposal. After disposing of the remaining filtered extract in the chemical waste container, filter approximately 50 ml of bleach through the filter containing the ground portion and allow to drain. Discard the filter paper and its contents (ground portion) into a plastic garbage bag for disposal. The bleach rinse filtrate collected may be treated as a non-hazardous solution and disposed of by pouring down the drain.

10.9 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits.

(1) 48 antibody-coated wells.

(2) 48 red-marked mixing wells.

(3) 1 yellow-labeled bottle of 1.5 ml 20 ppb aflatoxin control.

(4) 1 blue-labeled bottle of 7 ml aflatoxin-HRP conjugate solution.

(5) 1 green-labeled bottle of 24 ml K-blue substrate solution.

(6) 1 red-labeled bottle of 32ml red stop solution.

b. Materials Required but not Provided.

(1) Methanol - ACS grade or better.

(2) Deionized or distilled water.

(3) 250 ml graduated cylinder.

(4) Whatman 2V folded or S&S 24 cm pleated (or equivalent) filter paper.

(5) Filter funnel.

(6) Sample collection tubes.

(7) Blender with mixing jars.

(8) Balance.

(9) Sample grinder.

(10) Bio Tek EL 301 Microwell strip reader with 650 nm filter.

(11) 12-channel pipettor.

(12) 100 µl pipet.

(13) Pipette tips.

(14) Microwell holder.

(15) Waterproof marker.

(16) 2 reagent boats (to hold substrate and red stop solutions).

(17) Timer.

10.10 STORAGE CONDITIONS

a. The kit is packaged in a sealed "foil bag" with a label indicating the lot number and expiration date.

b. Store test kits between 36^{o}- 46^{o}F when not in use. Avoid prolonged storage of kits at room temperature. Do not freeze test kits.

c. Bring kits up to room temperature 64^{o}- 86^{o}F prior to use.

d. Do not use kit components beyond their expiration date.

CHAPTER 11

MYCO✓ TEST KIT

11.1 GENERAL INFORMATION

The Myco✓ test is a competitive enzyme-linked immunosorbent assay that provides quantitative measurement for the presence of aflatoxin in select grains and commodities. **The test kit is limited to providing aflatoxin measurements between 5 – 80 ppb.** Accurate aflatoxin measurements above 80 ppb can be obtained by performing a supplemental analysis involving a diluted extract.

11.2 PREPARATION OF SOLUTIONS

a. Extraction Solution.

The extraction solvent used in the Myco✓ test method is a methanol/water (distilled or deionized) mixture consisting of 70 percent methanol (ACS grade or better) and 30 percent water.

(1) Using a graduated cylinder, measure 700 ml of methanol and place it into a clean carboy with spigot.

(2) Add 300 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed.

(3) Label the container stating the mixture (70 percent methanol and 30 percent water), date of preparation, and initials of technician who prepared the solution.

(4) Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 7 parts methanol to 3 parts of deionized or distilled water.

b. Wash Solution.

(1) Transfer the contents of the Wash Concentrate vial to a 500-ml plastic squeeze bottle and add 475 ml of distilled or deionized water.

(2) Swirl to mix.

11.3 EXTRACTION PROCEDURES

a. Place a sheet of filter paper (Whatman #1 folded or equivalent) into a funnel mounted over a clean collection container.

b. Label the collection container with the sample identification.

c. Transfer 50 grams of ground sample into an extraction mixing jar.

d. Add 250 ml of the (70/30) methanol/water extraction solvent.

e. Cover the extraction jar and blend on high speed for 2 minutes.

f. Allow the extract to stand for 2-3 minutes to allow the slurry to settle.

g. Filter a minimum of 15 ml of the extract into the collection container.

11.4 TEST PROCEDURES

a. Allow reagents, antibody-coated wells, mixing wells, and sample extracts to reach room temperature prior to running the test.

b. Place the appropriate number of red mixing wells and clear test wells into a microwell holder.

NOTE: The maximum number of test samples that can be run at one time is 19. Using a strip of 12 wells, designate 5 wells for the calibrators and the remainder of the wells for test samples.

c. Using a pipette, dispense 150 µl of Enzyme Conjugate into each red mixing well.

d. Dispense 50 µl of each calibrator and sample into the appropriate red mixing wells using an adjustable or fixed 50 µl pipette.

NOTE: Use a clean pipette tip for each addition.

	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12
mixing wells	O	O	O	O	O	O	O	O	O	O	O	O
	C0	C10	C20	C40	C80	S1	S2	S3	S4	S5	S6	S7

Where C0 is the zero control, C10 is the 10 ppb control, C20 is the 20 ppb control, C40 is the 40 ppb control, and C80 is the 80 ppb control. S1 is sample 1, S2 is sample 2, S3 is sample 3, etc.

e. Using a multi-channel pipette, mix the contents of the wells by repeatedly filling and emptying the tips into the mixing wells.

f. Using a multi-channel pipette, transfer 100 µl of each reaction mixture directly into the corresponding clear test wells. Discard the mixing wells into an appropriate waste container.

g. Let the reaction mixture incubate for **exactly 5 minutes**. Mix the solution in the wells by gently swirling the plate on a flat surface for the first 15 seconds.

h. At the end of the 5-minute incubation period, dump the contents of the wells into an appropriate waste container. Using a 500-ml squeeze bottle containing wash solution, vigorously wash each well by overfilling. Repeat the vigorous wash for a **total of four washes**.

i. After the last wash, invert the wells and tap on absorbent paper to remove residual wash solution. Wipe excess liquid from the bottom of the wells.

j. Pour substrate solution into a clean reagent reservoir.

k. Dispense 100 µl of substrate solution into each test well using a multi-channel pipette.

l. Let the substrate solution incubate for **exactly 5 minutes**. Mix the solution in the wells by gently swirling the plate on a flat surface for the first 15 seconds.

m. Pour stop solution into a clean reagent reservoir.

n. Dispense 100 µl of stop solution into each test well using a multi-channel pipette.

o. Read and record the optical density of the wells at 650 nm using a Hyperion MicroReader™ 3 well reader. Make sure that the well bottoms are clean and dry before placing in the reader. Read the test results within 20 minutes of test completion. Use the data reduction software provided by SDI to quantify results.

11.5 REPORTING AND CERTIFYING TEST RESULTS

a. Report all results on the pan ticket and the inspection log to the nearest whole ppb.

b. Sample results over 80 ppb are reported as >80 ppb unless a supplemental analysis is performed.

c. Refer to the Certification section of the handbook for more detailed certification procedures.

11.6 SUPPLEMENTAL ANALYSIS

a. Diluting the Sample Extract.

If quantitative results are above the testing limits (i.e., 80 ppb) of the test kit, test results are reported as exceeding the limit. To determine and report an aflatoxin level higher than 80 ppb, the sample extract must be diluted so that a value between 5 and 80 ppb is obtained.

The final aflatoxin concentration is calculated by multiplying the results with the diluted extract by the dilution factor.

b. Example.

If the original analysis reported the aflatoxin value at 100 ppb, the sample extract would be diluted using the following procedures in order to obtain a true value.

(1) Dilute 5 ml of the original extract with 5 ml of the extraction solvent mixture. The total volume is 10 ml. This is a 1 to 2 dilution (compares volume in the beginning with the total volume in the end).

(2) Multiply the analytical results obtained by 2 to obtain the actual aflatoxin concentration. For example, if 54 ppb was the original value obtained with the diluted extract, the actual concentration in the original sample was 108 ppb.

$$\text{True Aflatoxin Value} = \frac{\text{Total Volume}}{\text{Initial Extract Volume}} \times \text{Aflatoxin Result}$$

$$\text{True Aflatoxin Value} = (10 \div 5) \times 54 \text{ ppb}$$
$$= 2 \times 54 \text{ ppb} = 108 \text{ ppb}$$

11.7 CLEANING LABWARE

a. Negative Tests (≤ 20 ppb).

(1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used glassware, funnels, beakers, etc., wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Place materials in a garbage bag for routine trash disposal.

b. Positive Tests (> 20 ppb).

(1) Labware.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water (e.g., 100 ml bleach to 1,000 ml water). Completely submerge the used glassware, funnels, beakers, etc., and soak for at least 5 minutes. Remove items from the bleach/water solution, submerge in a dishwashing liquid/water solution, wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water in a plastic pail labeled "bleach solution". Soak disposable materials, such as used columns, cuvettes, vials, test kit components, etc., for at least 5 minutes. Pour the liquid down the drain and place the materials in a garbage bag and discard.

11.8 WASTE DISPOSAL

a. Negative Results (≤ 20 ppb).

If the test result is negative (equal to or less than 20 ppb), discard the filter paper and its contents (ground material) into a plastic garbage bag for disposal. Dispose of any remaining liquid filtrate in the chemical waste container.

b. Positive Results (> 20 ppb).

If the result is positive (more than 20 ppb), the ground portion remaining in the filter paper must be decontaminated prior to disposal. After disposing of the remaining filtered extract in the chemical waste container, filter approximately 50 ml of bleach through the filter containing the ground portion and allow to drain. Discard the filter paper and its contents (ground portion) into a plastic garbage bag for disposal. The bleach rinse filtrate collected may be treated as a non-hazardous solution and disposed of by pouring down the drain.

11.9 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits

(1) 48 antibody-coated microtiter wells.

(2) 48 red-marked mixing wells.

(3) 5 vials each containing 2 ml of 0, 10, 20, 40, and 80 ppb of aflatoxin calibrators.

(4) 1 vial containing 8 ml of aflatoxin-HRP enzyme conjugate.

(5) 1 vial containing 8 ml of substrate.

(6) 1 vial containing 8 ml of stop solution.

(7) 1 vial containing 25ml of 20X wash concentrate.

(8) 4 multi-channel pipette reservoirs.

b. Materials Required but not Provided.

(1) Methanol - ACS grade or better.

(2) Deionized or distilled water.

(3) 100 ml graduated cylinder.

(4) Whatman #1 filter paper or equivalent.

(5) Glassware with 125 ml capacity for sample extraction.

(6) Filter funnel.

(7) 50 µl pipette with disposable tips.

(8) 50 -200 µl multi-channel pipette.

(9) 500 ml plastic squeeze bottle.

(10) Blender with mixing jars.

(11) Balance.

(12) Sample grinder.

(13) Hyperion MicroReader™ 3 Model 4027-002 with 650 nm filter.

(14) Timer.

(15) Waterproof marker.

(16) Microwell holder.

11.10 STORAGE CONDITIONS

a. Store test kits between 36^{o}- 46^{o} F when not in use. Avoid prolonged storage of kits at room temperature. Do not freeze test kits.

b. Do not use reagents from other SDI aflatoxin kits with different lot numbers.

c. Bring kits up to room temperature 64^{o}- 86^{o} F prior to use.

d. Do not use kit components beyond their expiration date.

U.S. DEPARTMENT OF AGRICULTURE
GRAIN INSPECTION, PACKERS AND STOCKYARDS ADMINISTRATION
FEDERAL GRAIN INSPECTION SERVICE
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CHAPTER 12

RIDASCREEN® FAST AFLATOXIN SC TEST KIT

12.1 GENERAL INFORMATION

The RIDASCREEN® FAST Aflatoxin SC test is a competitive enzyme immunoassay for the quantitative analysis of aflatoxin in select grains and commodities. **The test kit is limited to providing aflatoxin measurements between 5 – 100 ppb.**

12.2 PREPARATION OF SOLUTIONS

a. Extraction Solution.

The extraction solvent used in the RIDASCREEN® FAST Aflatoxin SC test is a methanol/water (distilled or deionized) mixture consisting of 70 percent methanol (ACS grade or better) and 30 percent water.

(1) Using a graduated cylinder, measure 700 ml of methanol and place it into a clean carboy with spigot.

(2) Add 300 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed.

(3) Label the container stating the mixture (70 percent methanol and 30 percent water), date of preparation, and initials of technician who prepared the solution.

(4) Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 7 parts methanol to 3 parts of deionized or distilled water.

b. Wash Solution.

(1) Dissolve the contents of the packet containing the buffer salt in 1 liter of distilled water.

(2) Swirl to mix.

(3) Store this solution in a refrigerator until needed. The solution expires 4 weeks after preparation.

12.3 EXTRACTION PROCEDURES

a. Transfer 50 grams of ground sample into an extraction mixing jar.

b. Add 250 ml of the (70/30) methanol/water extraction solvent.

c. Cover the extraction jar and blend on high speed for 2 minutes.

d. Filter approximately 1.5 ml of the extract through a filtering syringe or equivalent.

e. Dilute 1 ml of the filtrate with 1 ml of distilled or deionized water.

f. Proceed to test procedures.

12.4 TEST PROCEDURES

a. Sample Analysis.

(1) Allow reagents, microwells, and sample extracts to reach room temperature prior to running the test.

(2) Insert a sufficient number of wells into the microwell holder for all standards and samples to be tested. (For example: to test 7 samples use 8 wells - 1 for the standard and 7 for the test samples).

Test Strip

Well #	1	2	3	4	5	6	7	8
Sample	C 0	S1	S2	S3	S4	S5	S6	S7

Where C 0 is the zero control, S1 is sample 1, S2 is sample 2, S3 is sample 3, etc.

NOTE: Do not run more than 3 strips (23 samples) per set of control standards.

(3) Using a new pipette tip for each standard and sample, pipet 50 µl of standard and prepared sample to separate wells.

(4) Add 50 µl of enzyme conjugate (red capped bottle) into each well.

(5) Add 50 µl of anti-aflatoxin antibody (black capped bottle) into each well.

(6) Mix thoroughly by gently sliding the plate back and forth on a flat surface.

(7) Incubate for 10 minutes (± 1.0 minutes) at room temperature (64 – 86º F).

(8) Dump the contents of the wells. Turn the wells upside down and tap out on a paper towel until the remaining liquid has been removed.

(9) Using a wash bottle, fill each well with distilled or deionized water or washing buffer solution. Empty the wells again and remove all remaining liquid. Repeat this step 2 times (total of 3 washes).

(10) Add 100 µl of substrate/chromagen (white dropper bottle) to each well.

(11) Mix thoroughly by gently sliding the plate back and forth on a flat surface.

(12) Incubate for 5 minutes (± 0.5 minutes) at room temperature (64 – 86º F). Cover the wells with a paper towel to protect them from light sources.

(13) Add 100 µl of stop solution (yellow or orange dropper bottle) to each well.

(14) Mix thoroughly by gently sliding the plate back and forth on a flat surface.

(15) Measure absorbance at 450 nm using the Biotek EL 301, or Awareness Technology Stat-Fax Model 303 PLUS microwell readers.

(Results must be read within 10 minutes)

b. Reading Results with the Microwell Reader.

(1) Biotek EL 301 Microwell Reader.

(a) Make sure that the microwell reader is on and allowed to warm-up for a minimum of 15 minutes before using.

(b) Remove sample carriage and hit "Enter."

(c) Insert W2 filter and hit "Enter."

(d) Insert W1 filter (450 nm) and hit "Enter."

(e) Hit "Clear" and then "Blank." This will cause the instrument to read air as the blank sample.

(f) Load microwells into sample carriage so that the first control labeled 0 is in position A1.

(g) Load the sample carriage into the strip reader so that position A1 is under the light beam of the reader.

(h) Press "Read" and an absorbance value for A1 should appear in the display on the microwell reader. Record the value.

(i) Slide the carriage to position A2 and press "Read." An absorbance value for A2 will appear. Record the value.

(j) Repeat step (i) until absorbance values have been obtained for the control and all samples. Record the values.

(k) Use the RIDA®SOFT Win Data software provided by r-Biopharm to convert the absorbance values into concentration values.

(2) Stat-Fax Model 303 PLUS Microwell Reader

(a) To begin from the "Ready" prompt, press Menu, key in the test number, and then press Enter.

(b) The screen will read, "Set carrier to A, press enter." Place the wells all the way to the right in the carrier. Push the carrier all the way to the left to line up the notch with the wells, then press enter. The carrier will advance into the reader, and it should start to print.

(c) When the reader is finished reading the strip, the screen will read, "Plot Curve Y/N?"

Press "Yes" (1/A) to print the graph,

Press "No" (0) to skip this feature.

(d) The screen will read, "Accept Curve Y/N ?"

Press "Yes" (1/A) to accept the curve and proceed to read another strip. When finished reading the second strip, press "Clear" twice and the results strip will print, "Test Ended."

Press "No" (0) to end the test.

12.5 REPORTING AND CERTIFYING TEST RESULTS

a. Report all results on the pan ticket and the inspection log to the nearest whole ppb.

b. Sample results over 100 ppb are reported as >100 ppb unless a supplemental analysis is performed.

c. Refer to the Certification section of the handbook for more detailed certification procedures.

12.6 SUPPLEMENTAL ANALYSIS

a. Diluting the Sample Extract.

If quantitative results are above the testing limits (i.e., 100 ppb) of the test kit, test results are reported as exceeding the limit. To determine and report an aflatoxin level higher than 100 ppb, the sample extract must be diluted so that a value between 5 and 100 ppb is obtained.

The final aflatoxin concentration is calculated by multiplying the results with the diluted extract by the dilution factor.

b. Example.

If the original analysis reported the aflatoxin value at 130 ppb, the sample extract would be diluted using the following procedures in order to obtain a true value.

(1) Dilute 5 ml of the original extract (obtained from step c., section 6.3) with 5 ml of the extraction solvent mixture. The total volume is 10 ml. This is a 1 to 2 dilution (compares volume in the beginning with the total volume in the end).

(2) Multiply the analytical results obtained by 2 to obtain the actual aflatoxin concentration. For example, if 74 ppb was the original value obtained with the diluted extract, the actual concentration in the original sample was 148 ppb.

$$\text{True Aflatoxin Value} = \frac{\text{Total Volume}}{\text{Initial Extract Volume}} \times \text{Aflatoxin Result}$$

$$\text{True Aflatoxin Value} = (10 \div 5) \times 74 \text{ ppb}$$
$$= 2 \times 74 \text{ ppb} = 148 \text{ ppb}$$

12.7 CLEANING LABWARE

a. Negative Tests (≤ 20 ppb).

(1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used glassware, beakers, etc., wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Place materials in a garbage bag for routine trash disposal.

b. Positive Tests (> 20 ppb).

(1) Labware.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water (e.g., 100 ml bleach to 1,000 ml water). Completely submerge the used glassware, beakers, etc., and soak for at least 5 minutes. Remove items from the bleach/water solution, submerge in a dishwashing liquid/water solution, wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water in a plastic pail labeled "bleach solution". Soak disposable materials, such as used columns, cuvettes, vials, test kit components, etc., for at least 5 minutes. Pour the liquid down the drain and place the materials in a garbage bag and discard.

12.8 WASTE DISPOSAL

a. Negative Results (≤ 20 ppb).

If the test result is negative (equal to or less than 20 ppb), discard the syringe into a plastic garbage bag for disposal.

b. Positive Results (> 20 ppb).

If the result is positive (more than 20 ppb), the remaining ground portion must be decontaminated, using bleach, prior to disposal. Discard the filter syringe and remaining ground portion into a plastic garbage bag for disposal. The bleach rinse filtrate collected may be treated as a non-hazardous solution and disposed of by pouring down the drain.

12.9 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits

(1) 1 microtiter plate.

(2) 48 antibody coated microwells.

(3) 1 aflatoxin standard solution of 1.3 ml of 0 ppb aflatoxins.

(4) 1 red-capped bottle of 3 ml peroxidase conjugated aflatoxin solution.

(5) 1 black-capped bottle of 3 ml anti-aflatoxin antibody.

(6) 1 white dropper bottle of 6 ml Substrate/Chromagen.

(7) 1 yellow or orange dropper bottle of Stop reagent.

(8) 1 washing buffer.

b. Materials Required but not Provided:

(1) Methanol - ACS grade or better.

(2) Deionized or Distilled Water.

(3) 250 ml graduated cylinder.

(4) 125 ml container.

(5) Filtering syringe (JM1000), Whatman No. 1 filter paper, or equivalent.

(6) Sample collection tubes.

(7) Waring high-speed blender with a one liter jar, or equivalent.

(8) Sample grinder.

(9) Balance.

(10) Biotek EL 301 or an Awareness Technology Inc. Stat-Fax Model 303 Plus Microwell reader equipped with a 450-nm filter.

(11) Eppendorf Repipettor, or equivalent, and 2.5 ml syringes.

(12) 50 µl, 100 µl, and 1000 µl pipettor and pipette tips.

(13) Paper towels, Kaydry paper or equivalent absorbent material.

(14) Waste receptacle.

(15) Timer: 3 channel minimum.

(16) Waterproof marker, Sharpie or equivalent.

(17) Wash bottle.

12.10 STORAGE CONDITIONS

a. Storage Conditions.

(1) The reagents supplied with the test kit can be used until the expiration date on the kit label when stored refrigerated at temperatures between 35° F and 46° F. **(DO NOT FREEZE)**

(2) Return any unused microwells to their original foil bag and reseal them together with the desiccant provided.

(3) The substrate/chromogen solution is light sensitive, therefore, avoid exposure to direct light.

b. Indication of Instability or Deterioration of Reagents.

(1) Any bluish coloration of the red stained substrate/chromogen solution is indicative for deterioration and the reagent should be discarded.

(2) A value of less than 0.6 absorbance units for the zero standard may indicate deterioration of reagents.

U.S. DEPARTMENT OF AGRICULTURE
GRAIN INSPECTION, PACKERS AND STOCKYARDS ADMINISTRATION
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CHAPTER 13

QUICKTOX TEST KIT

13.1 GENERAL INFORMATION

The QuickTox test kit uses lateral flow test strip technology that provides qualitative (equal to or less than a specified threshold) results.

13.2 PREPARATION OF EXTRACTION SOLUTION

The extraction solvent used in the QuickTox test method is a methanol/water (distilled or deionized) mixture consisting of 70 percent methanol (Reagent grade or better) and 30 percent water.

a. Using a graduated cylinder, measure 700 ml of methanol and place it into a clean carboy with spigot.

b. Add 300 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed.

c. Label the container stating the mixture (70 percent methanol and 30 percent water), date of preparation, and initials of technician who prepared the solution.

d. Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 7 parts methanol to 3 parts of deionized or distilled water.

13.3 EXTRACTION PROCEDURES

a. Transfer 50 grams of ground sample into an extraction mixing jar.

b. Add 100 ml of the (70/30) methanol/water extraction solvent.

c. Cover the extraction jar and shake by hand for 2 minutes. If a mechanical shaker is used shaking time may be reduced to 1 minute.

d. After shaking, the sample will immediately begin to separate into 2 layers. The top (yellowish) layer containing the aflatoxin residues will be used for testing.

13.4 TEST PROCEDURES

a. Reaction Vial.

(1) Using the fixed volume pipette included in the test kit, place 150 microliters (150 µl) **tap** water into a reaction vial.

(2) Using the same fixed volume pipette, remove 150 µl from the top (yellowish) layer of the extract. Add the extraction solution to the reaction vial containing water.

(3) Mix water and sample extraction solution by stirring with the tip of the fixed volume pipette.

NOTE: To ensure correct volumes are used to prepare the test sample, a fixed volume pipette is included with the kit. When a liquid drawn to the top of the straw end of the pipette is dispensed, 150 µl will be expelled into the reaction vial. Any overfill is retained in the pipette. After diluting the sample the final volume in the reaction vial should be 300 µl. Do not reuse diluted samples. Use a new fixed volume pipette and reaction vial for each sample.

b. Test Strips.

(1) Allow refrigerated canisters to come to room temperature before opening. Remove the QuickTox strips to be used then immediately reseal the canister. Avoid bending the strips.

(2) Place the strip into the reaction vial containing the diluted sample extract. The arrow tape on the end of the strip should point into the reaction vial.

(3) The sample extract will travel up the strip. Reaction vials will stand on their own or may be inserted into the cardboard racks provided.

(4) Allow the strip to develop for 5 minutes before making final assay interpretations. Negative sample results may become obvious more quickly (2 – 3 minutes).

c. Interpreting the Lateral Flow Test Strip.

Development of a Control Line within 5 minutes indicates that the strip has functioned properly. Any strip that does not develop a Control Line should be discarded. A second preparation of the extract (using a fresh 1:2 dilution) should be made and tested using another strip.

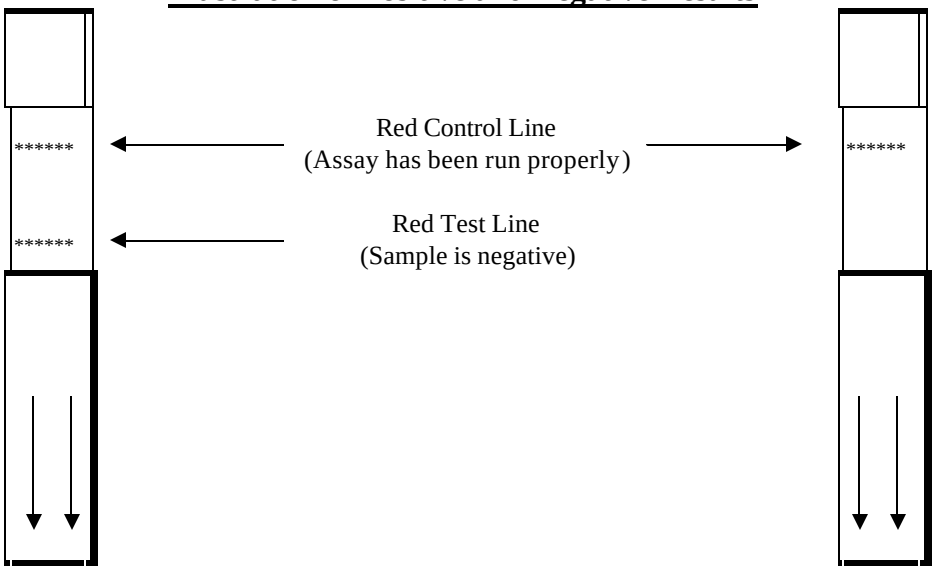
(1) Negative Result.

A sample containing aflatoxin residues less than or equal to 20 ppb will develop 2 distinct lines, the Control Line and the Test Line, in the test area.

(2) Positive Result.

A sample containing aflatoxin residues in excess of 20 ppb will develop 1 distinct line, the Control Line.

Illustration of Positive and Negative Results



13.5 REPORTING AND CERTIFYING TEST RESULTS

a. Report results on the pan ticket and inspection log as being equal to or less than 20 ppb (≤ 20 ppb), or as exceeding 20 ppb (> 20 ppb), as applicable.

b. Certify results as being equal to or less than 20 ppb or exceeding 20 ppb, as applicable.

c. Refer to the Certification section of the handbook for more detailed certification procedures.

13.6 CLEANING LABWARE

a. Negative Tests (≤ 20 ppb).

(1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used extraction mixing jars, wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Place materials in a garbage bag for routine trash disposal.

b. Positive Tests (> 20 ppb).

(1) Labware.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water (e.g., 100 ml bleach to 1,000 ml water). Completely submerge the used extraction mixing jars and soak for at least 5 minutes. Remove items from the bleach/water solution, submerge in a dishwashing liquid/water solution, wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water in a plastic pail labeled "bleach solution". Soak disposable materials, such as used test strips and pipettes, for at least 5 minutes.

Pour off the liquid down the drain and place the materials in a garbage bag and discard.

13.7 WASTE DISPOSAL

a. Negative Results (≤ 20 ppb).

If the test result is negative (equal to or less than 20 ppb), dispose of any remaining liquid filtrate in the chemical waste container. Discard the sample slurry (ground material) into a plastic garbage bag for disposal.

b. Positive Results (> 20 ppb).

If the result is positive (more than 20 ppb), the slurry (ground portion) remaining in the sample extraction jar must be decontaminated prior to disposal. After disposing of the remaining filtered extract in the chemical waste container, pour approximately 50 ml of bleach solution into the sample extraction jar and shake to mix with the sample slurry. After the slurry and bleach solution separate, handle the bleach rinse filtrate as a non-hazardous solution and dispose of by pouring the liquid down the down. Discard the sample slurry (ground portion) paper into a plastic garbage bag for disposal.

13.8 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits

(1) 50 QuickTox strips packed in a moisture-resistant container.

(2) 50 fixed volume transfer pipettes.

(3) 50 reaction vials.

b. Materials Required but not Provided:

(1) Timer (5 minute capacity).

(2) Felt tipped pens.

(3) Balance.

(4) Sample Grinder.

(5) Methanol - Reagent grade or better.

(6) Deionized or Distilled water.

(7) Sample extraction jars.

(8) Orbital/rotary shaker.

(9) Tap water.

13.9 STORAGE CONDITIONS

a. Storage Conditions.

Test kits should be refrigerated between 36°- 48°F.

b. Precautions.

(1) Do not use the test kits beyond the noted expiration date.

(2) Prolonged exposure to high temperatures may adversely affect the test results.

(3) Do not open the desiccated canister until ready to use the strips.

CHAPTER 14

AFLACARD T20 TEST KIT

14.1 GENERAL INFORMATION

The AFLACARD T20 test kit is a qualitative enzyme immunoassay procedure for the detection of total aflatoxins. The test provides qualitative (less than or equal to a specified threshold) results.

14.2 PREPARATION OF EXTRACTION SOLUTION

The extraction solvent used in the AFLACARD T20 test method is a methanol/water (distilled or deionized) mixture consisting of 80 percent methanol (Reagent grade or better) and 20 percent water.

a. Using a graduated cylinder, measure 800 ml of methanol and place it into a clean carboy with spigot.

b. Add 200 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed.

c. Label the container stating the mixture (80 percent methanol and 20 percent water), date of preparation, and initials of technician who prepared the solution.

d. Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 8 parts methanol to 2 parts of deionized or distilled water.

14.3 PREPARATION OF TESTING MATERIALS

a. Conjugate.

(1) Add 2 ml of conjugate diluent buffer (pink label) to the freeze-dried conjugate (amber vial).

(2) Replace the rubber cap and mix gently by inversion.

(3) Transfer all of the conjugate into the empty conjugate dropper bottle (red label) and write the preparation date on the label.

(4) **Leave the conjugate at room temperature for at least 30 minutes before use.**

NOTE: The ready to use conjugate is stable at 36° - 46° F

b. Other Kit Components.

(1) Remove the AFLACARD T20 kit from the refrigerator and leave the test kit components; substrate (blue label), wash buffer (green label), substrate (blue label), stop solution (yellow label), and test card at room temperature for at least 30 minutes before using the test.

Each card has two ports and therefore can perform two tests. The second port should be used within 8 hours of the first port. Each port has a sample area and control area. Please ensure the airholes on the card are on the right hand side and are not blocked or covered during the assay.

(2) Check that the two ports on the card to be used each exhibit two light blue spots.

NOTE: Each unused card has two light blue spots on each port which will disappear during the course of the assay.

14.4 EXTRACTION PROCEDURES

a. Transfer 50 grams of ground sample into an extraction mixing jar.

b. Add 100 ml of the (80/20) methanol/water extraction solvent.

c. Cover the extraction jar and blend on high speed for 1 minute.

d. Remove the cover and funnel a minimum of 10 ml of the extract through a Whatman No.4 filter paper into a sample jar labeled with the sample identification.

e. After collecting the filtrate, remove the funnel, filter, and ground material and place over an empty collection container.

14.5 TEST PROCEDURES

a. Sample Preparation.

(1) Remove the lid from the sample diluent tube and add 200 µl of filtrate.

(2) Cap and invert sample diluent tube. The sample is now ready to be applied to the card.

b. Sample Analysis.

(1) Apply 250 µl of diluted filtrate to sample port and incubate for a minimum of 1 minute. Always ensure that the liquid has passed completely through the membrane before proceeding to the next step.

(2) Using the conjugate dropper bottle (red label), apply 3 drops of conjugate to the test port and incubate for 1 minute.

(3) Using the wash buffer dropper bottle (green label), apply 3 drops of wash buffer to the test port and incubate for 1 minute.

(4) Dry around the port with a tissue.

(5) Using the substrate dropper bottle (blue label), apply 3 drops of substrate to the test port and incubate for 2 minutes.

(6) Using the stop solution dropper bottle (yellow label), apply 3 drops of stop solution to the test port. Allow the solution to pass completely through the membrane.

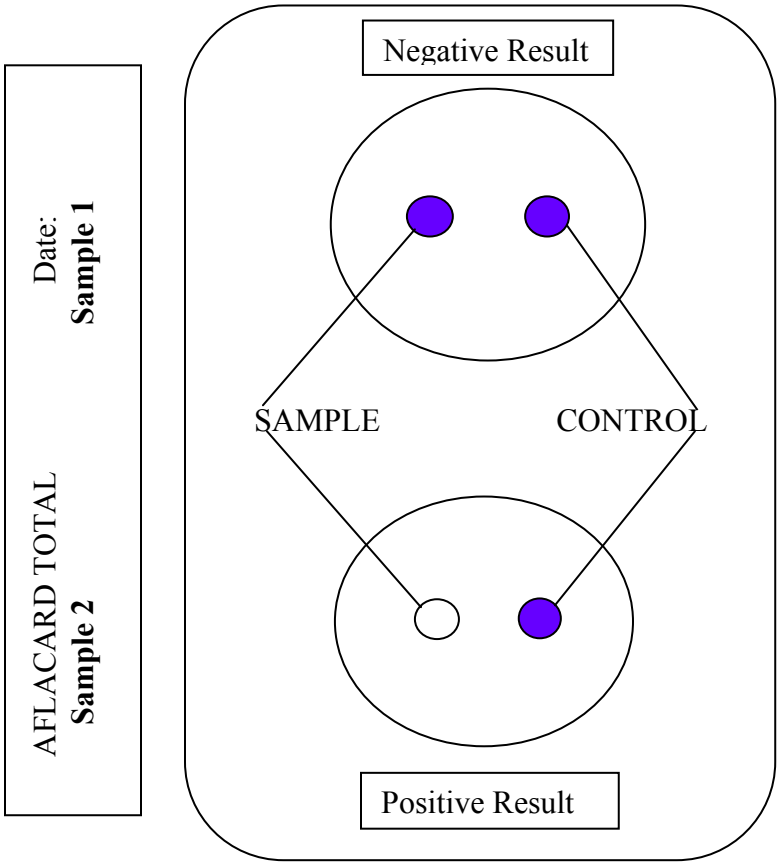
c. Reading Test Results.

(1) The control spot must develop a **clearly visible purple color** in order to have a valid test result. The color in the sample and the control spot does not need to be of the same intensity.

(2) The sample should be considered to be negative when the sample and control spot both have clearly visible color development.

(3) The sample should be considered to be positive (more than 20 ppb) when there is no detectable color on the sample spot.

INTERPRETATION OF RESULTS



14.6 REPORTING AND CERTIFYING RESULTS

a. Report results on the pan ticket and inspection log as being equal to or less than a threshold (e.g., 20 ppb) or as exceeding the threshold.

b. Certify results as being equal to or less than a threshold.

c. Refer to the Certification section of the handbook for more detailed certification procedures.

14.7 CLEANING LABWARE

a. Negative Tests (≤ 20 ppb).

(1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used glassware, funnels, beakers, etc., wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Place materials in a garbage bag for routine trash disposal.

b. Positive Tests (> 20 ppb).

Prepare a bleach solution consisting of 1 part bleach to 10 parts water (e.g., 100 ml bleach to 1,000 ml water). Completely submerge the used glassware, funnels, beakers, etc., and soak for at least 5 minutes. Remove items from the bleach/water solution, submerge in a dishwashing liquid/water solution, wash thoroughly, then rinse with clean water before reusing.

14.8 WASTE DISPOSAL

a. Negative Results (≤ 20 ppb).

If the test result is negative (equal to or less than 20 ppb), discard the filter paper and its contents (ground material) into a plastic garbage bag for disposal. Dispose of any remaining liquid filtrate in the chemical waste container.

b. Positive Results (> 20 ppb).

If the result is positive (more than 20 ppb), the ground portion remaining in the filter paper must be decontaminated prior to disposal. After disposing of the remaining filtered extract in the chemical waste container, filter approximately 50 ml of bleach through the filter containing the ground portion and allow to drain. Discard the filter paper and its contents (ground portion) into a plastic garbage bag for disposal. The bleach rinse filtrate collected may be treated as a non-hazardous solution and disposed of by pouring down the drain.

14.9 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits:

(1) 10 Aflacard Total Cards.

(2) 20 tubes containing 3.8 ml Sample Diluent Buffer.

(3) 2 Freeze-dried Conjugate vials (red label).

(4) 1 Conjugate Diluent Buffer vial (pink label).

(5) 2 Conjugate Dropper Bottles (empty, red label).

(6) 1 Wash Buffer dropper bottle (green label).

(7) 1 Substrate dropper bottle (blue label).

(8) 1 Stop Solution dropper bottle (yellow label).

b. Materials Required but not Provided:

(1) Sample grinder.

(2) Balance.

(3) Methanol - ACS grade.

(4) Distilled or deionized water.

(5) Blender with mixing jars.

(6) Timer.

(7) Whatman No.4 Filter Paper.

(8) Tissue paper.

(9) Sample collection container.

14.10 STORAGE CONDITIONS

a. Storage Conditions.

Test kits should be refrigerated between 36° - 46° F. **Do not freeze.**

b. Precautions.

(1) Do not use kit components beyond the expiration date.

(2) Do not use reagents from one batch number in conjunction with reagents from a different batch number, and do not substitute reagents from other manufacturers.

(3) Kits should be brought to room temperature (68° - 82° F) prior to use. This will take approximately 30 minutes.